

**Theme:**  
**Catheterisation.**  
**Read the 3**  
**informative**  
**articles**

*page 6*

- Group housing vs single housing? *page 23*
- Prolonged anaesthesia in minipigs *page 11*
- The Minipig in Preclinical Development – a Modelling and Simulation Perspective *page 4*
- Challenges for detecting the end of cardiac repolarization in the ECG from Göttingen minipigs *page 18*
- Experiences from using the Göttingen Minipig as a model of human genital Chlamydia infection *page 16*

**Lars Ellegaard  
has been  
awarded!**

*page 2*



Clean pigs  
for clear results

## Dear Reader

You might not recognise this newsletter, as it introduces our new logo. For the past eight years, our logo has been the easily recognizable drawing of a minipig. During these years, Ellegaard Göttingen Minipigs has grown considerably and the Göttingen Minipig has become a well-known, commonly-used, non-rodent model in biomedical research. No longer do we need to draw and define the Göttingen Minipig, so based on this development, we have created a new logo. The new logo is straightforward, strong, harmonious, coherent and scientific – just like our company. Our new logo has a link to our previous logo and therefore to our history – Do you see it?

2012 was another interesting and promising year for our company and the Göttingen Minipig. We are pleased to see how the minipig is being selected for an increasing number of studies and that its market share of non-rodents in biomedical research has grown significantly. Therefore we still see great prospects for the Göttingen Minipig in the pharmaceutical industry. The demand for minipigs is not just growing in Europe but also in North America and Japan. Marshall BioResources is expanding its minipig facilities in Upstate New York to meet the rising demand, and 2013 will be the year when the Göttingen Minipig will be a locally bred research model in Japan.

For a couple of years we have been shipping minipigs to Oriental Yeast Co. for redistribution to Japanese pharmaceutical companies and CROs, but soon Göttingen Minipigs will be bred and distributed directly from Oriental Yeast Co.'s site in Ina, Japan. Oriental Yeast Co. has completed the building of a new barrier facility for minipigs and in the spring of 2013 we will send a breeding herd to Japan.

We look forward to continuing the good collaboration with our license partners in USA and Japan and to exploring their markets further.

---

## Gold Medal for Lars Ellegaard

At a ceremony in December 2012, the founder of Ellegaard Göttingen Minipigs, Lars Ellegaard, was awarded the University of Göttingen's gold medal – Aureus Gottingensis.

*“With this award the Senate and the Presidential Board would like to honour you for your extraordinary merit concerning the Göttingen Minipig. With the development and marketing of the Minipig you have been contributing to the international reputation of our University for more than two decades.”*

Since 1965 the Gold Medal has been awarded to five people besides Lars Ellegaard and the award is an outstanding recognition of Lars Ellegaard's efforts to transform a scientific university project into a commercial success of importance to the University of Göttingen and to the entire pharmaceutical industry.

Lars Ellegaard is enjoying his well-deserved retirement and appreciates this acknowledgement from the University of Göttingen.

In December 2012, Lars Ellegaard was awarded the University of Göttingen's gold medal – Aureus Gottingensis for his efforts of making the Göttingen Minipig known worldwide, hence disseminating the name of the University in Göttingen. Lars created an excellent foundation for Ellegaard Göttingen Minipigs, and we acknowledge his importance not only to the University of Göttingen and our company, but to the entire biomedical industry. We have always had good collaboration with many customers, and a lot of background data and new knowledge have emerged from such collaborations. In 2013, we would like to launch a new initiative which will make even closer cooperation with CROs possible. Further information about this opportunity can be found in this newsletter.

An increasing amount of background data and knowledge relating to the Göttingen Minipig is available which makes this species comparable to other non-rodent species. The selection of an animal model for a study should be based on science, not habit. Comparing the available animal models before a study and selecting the most suitable model, will make the results the clearest and reduce the risk of having to redo the study. Thus, meticulously selecting the proper species can contribute to the 3Rs as well as to the corporate social responsibility of the company and the entire industry.

Based on this, we have rephrased our vision: *The Göttingen Minipig should always be included as an option on equal terms with other non-rodent species in the selection of the most appropriate species for a study.*

I hope you will enjoy reading this newsletter, which includes many interesting articles.

*Sincerely,  
Jens Ellegaard, CEO*



Prof. Henner Simianer and President Prof. Ulrike Beisiegel from the Georg-August University in Göttingen at the ceremony where Lars Ellegaard was awarded the gold medal.

*“The RETHINK project brought me great experience with good friends and scientists. To top this off by receiving the Aureus Gottingensis Medal was a great pleasure for me and my family and I thank the University and those who decided to reward me with this distinction.”*

# CRO Cooperation

We envision that the Göttingen Minipig should always be an option on equal terms with other non-rodent species in the selection of the most appropriate species for a study.

Species selection should not be based on tradition and habits, but science: the Göttingen Minipig should be selected for a study whenever it is the most suitable model. We do our best to disseminate these messages to help our customers by giving them the best opportunities to achieve the clearest scientific results. With this in mind, we continuously focus on developing new background data, supporting validation studies and cooperating with our customers to secure a fully updated knowledge database where all relevant information about the Göttingen Minipig is available.

Much knowledge relating to the Göttingen Minipig is being published and we always find it interesting to review articles, posters, meeting abstracts and other material which includes knowledge and experiences from minipig users around the world. This kind of knowledge sharing is invaluable in the process of putting the Göttingen Minipig in the spotlight within the pharmaceutical industry.

We appreciate the good collaboration we have with many customers and we would like to commence a new initiative which will make even closer cooperation with CROs possible.

It is up to every CRO to decide whether to initiate this closer cooperation with us and you are welcome to contact us if you would like to have a discussion about this opportunity.

## Partnering for success in preclinical development

The idea underlying the concept is to make a common effort to develop and disseminate knowledge about the Göttingen Minipig. We propose cooperation based on training and support on our part, as well as on development and promotion on the part of each CRO.

We would like to help to emphasise and further develop the specific strengths and fields of specialisation for each CRO who enters into closer cooperation with us. In relation to this we would like to assist CROs in preparing, creating and distributing relevant scientific material and to ensure more face-to-face time with prospective customers.

We encourage any CRO who is interested in this closer cooperation to contact us for further information. The cooperation proposed has no fixed framework but can be adapted to our mutual interests.



## New Area Sales Manager

In November 2012, Niels Thun Andersen started at Ellegaard as Area Sales Manager, Europe.

Niels duties will involve visiting existing and prospective customers in Europe to provide information and updates on the Göttingen Minipig.

Niels has an MSc in economics and business administration and has international sales experience from the pharmaceutical and life science industry, where he has worked for LEO Pharma, Dako (diagnostics) and Ambu (medical devices).

Niels has wide international experience in building up and managing sales organisations and working with distributors and partners in Asia, the Middle East and Africa. He has been living in Asia for 10 years where he met his wife, and they have 2 sons. Most of his leisure time is spent with his family in their holiday cottage.

We hope that you will have the opportunity to meet Niels and discuss your future work with minipigs. You are welcome to contact him to request a visit.

## CONTENTS

Gold Medal for Lars Ellegaard . . . . .	2
New Area Sales Manager . . . . .	2
CRO Cooperation . . . . .	3
The 2012 meeting of the Minipig Research Forum. . . . .	3
The Minipig in Preclinical Development - a Modelling and Simulation Perspective . . . . .	4
Catheter Workshop & Surgery course . . . . .	5
Catheters for Vascular Access and the Göttingen Minipig . . . . .	6
Evaluation of blood sampling through a venous catheter - Seldinger Technique. . . . .	9
Refinement: Implantation of Central Venous Catheter in Minipigs. . . . .	10
Prolonged Anaesthesia in Minipigs . . . . .	11
Experiences from using the Göttingen Minipig as a model of human genital chlamydia infection . . . . .	16
Challenges to detecting the end of cardiac repolarisation in the Göttingen Minipig ECG. . . . .	18
Minipig Activity - group housing vs single housing . . . . .	23
SOT Exhibitor Sponsored Sessions . . . . .	24
Reviews of the book "The Minipig in Biomedical Research . . . . .	24
Meeting calendar . . . . .	24

# The Minipig in Preclinical Development – a Modelling and Simulation Perspective

## Authors:

Dr Claudia Suenderhauf, Neil Parrott, Georg Schmitt, Dr Thierry Lave and Dr Thomas Singer

*Modelling & Simulation, Dept. of Project Leaders/M&S, Non-Clinical Safety, pRED, F. Hoffmann-La Roche AG, CH-4070 Basel, Switzerland*

Decision-taking during preclinical development at Roche is strongly assisted by modelling and simulation. This means that we systematically apply pharmacokinetic and pharmacodynamic (PK/PD) modelling to interpret pharmacological and toxicological animal studies. Simulation of pharmacokinetic profiles enables us to select meaningful dose levels as well as optimal sampling time points for an efficient study design. This can enhance the drug-development process considerably as whole study arms with inappropriate exposures can be avoided. This strategy is a strong contribution to our continuous efforts to be at the forefront of refining, reducing and replacing animal experiments (3 Rs) by means of innovative techniques.

Besides the study design, the extrapolation from in vitro data and in vivo animal data to clinical outcomes, even at a very early stage of development, is a key strength of the modelling approach. At Roche we apply physiologically based pharmacokinetic (PBPK) models to translate preclinical pharmacokinetic readouts into the clinic. These mechanistic models use knowledge on the physiology and the compound under investigation to predict pharmacokinetic profiles. PBPK models map compartment sizes to actual, measured organ or tissue volumes and transfer rate constants between the compartments corresponding to blood flows. The approach also incorporates physicochemical properties of the compound under investigation, like measures of lipophilicity and charge to describe drug partitioning into blood, plasma and tissues. A key strength of PBPK models is that they can be updated and refined as knowledge on the drug candidate grows during development. Moreover, the models can be translated from one species to another. In this sense, the model can be seen as a knowledge base for the compound behaviour, which will be finally used to transfer to humans and predict human safety windows.

In contrast to more empirical approaches, no pharmacokinetic data is needed to perform a preliminary prediction, which is a particular advantage at very early stages of drug development. However, the creation of a PBPK model is more time-consuming than fitting a compartment model: It needs an extensive knowledge base on the physiology of each species and benefits from validation and refinement on several test compounds. For traditionally used laboratory species such as mouse, rat, and dog,

physiological databases have been compiled and PBPK models established and applied.

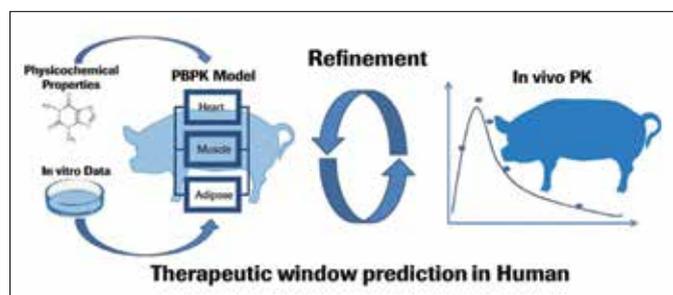
The minipig is an innovative disease model, which is becoming more and more appreciated as well-suited for preclinical development. As the minipig shares many physiological and anatomical features with human beings, it can be more predictable for human safety than other, more established laboratory species. In this way, a scientifically driven selection of the minipig can help to reduce testing in non-responder species (3 Rs).

To embed the minipig successfully into the implemented work flow, including supportive modelling and simulation, we implemented a new PBPK model for the species as no model has been published so far.

The first step for the model creation was screening the public domain as well as in house databases to assemble a data compilation on minipig physiology. We were particularly interested in accurate information on tissue volumes and perfusion as well as absorption-relevant parameters. As the minipig is a fast-growing species, we restricted our model to the physiology of a 6-month-old male, which resembles the standard animal size in toxicity studies.

For most organ systems, the information was well-established. However, some particular areas needed closer investigation. Especially diffuse tissues such as skin, muscle and adipose are difficult to measure experimentally but are crucial for drug distribution due to their size and constitution. In these cases, several data sources had to be compared and challenged with our knowledge from other species. For skin, to mention an example, we achieved a reasonable approximation by calculating body surface area and multiplying it by histologically-measured average skin thickness. Such assumptions on the distribution model were successfully verified by reproducing reference compound concentration time profiles from intravenous PK studies in the minipig.

Another challenging area of the minipig model was the parameterisation of intestinal absorption. In the standard preclinical setting, candidate drugs are administered orally as solutions, suspensions or capsules and therefore PBPK modelling of stomach passage and intestinal absorption is essential. It can generally be stated that the absorption process is complex and involves factors such as drug partitioning in micelles/lipid vesicles, chemical and enzymatic stability in the lumen, drug precipitation, gut metabolism and active transport. Several PBPK absorption models incorporating these factors have been defined for human and laboratory species and we followed these examples by collecting data for minipig intestinal surface area, residence time, fluid volumes and regional pH. The characterisation of the gastrointestinal system of pigs and minipigs was addressed by several workgroups, and in general the species closely resembles human gastrointestinal physiology. However,



on some parameters, such as stomach pH and transit times, study results from the public domain exhibited a broad distribution, which was difficult to interpret. Moreover, information on minipig bile-salt concentrations, which would be particularly valuable for absorption prediction of poorly soluble compounds, was not available to us.

Despite some gaps in our minipig knowledge base, our preliminary PBPK model performed encouragingly in a first validation exercise. It is clear that more research work has to be invested, especially for better understanding of the gastrointestinal physiology in minipigs. More in-depth studies are currently ongoing at Roche.

Another important part of the puzzle will be the incorporation of clearance estimates from in vitro experiments in minipig hepatocytes and microsomes. These are currently the gold standard for predicting metabolic clearance. Scaling factors will be needed for translation from in vitro to in vivo as has been done for other laboratory species and human.

In this way, our future work will not only aim at establishing a state-of-the-art PBPK model, but also close relevant existing gaps in our knowledge on minipig physiology. These have to be addressed if the species is to be successfully deployed in preclinical research. We feel confident that with increasing experience and knowledge, we will be able to address these issues, and the preliminary PBPK model will be a good basis on which to test and verify our results.

The current version of the minipig PBPK model discussed here and the corresponding minipig database were published in *Pharmaceutical Research* early this year (*Pharm Res.* 2013 Jan; 30(1):1-15).

## The 2012 meeting of the Minipig Research Forum

In November 2012, the sixth annual meeting of the Minipig Research Forum attracted 60 minipig users to Frankfurt, Germany. The venue was the Jumeirah Hotel which created nice surroundings for the interesting 1½ day meeting.

The programme for the meeting focused mainly on study planning, conduct and assessment, and 16 speakers presented information and experiences from studies which included minipigs.

For the first time the MRF meeting included workshops where the attendees were divided into groups and shared their knowledge and experiences about minipig health & welfare, clinical observations & humane endpoints in minipig safety studies and minipig ophthalmology. The workshops also enabled discussions of other relevant topics, and the meeting in general was a good opportunity for minipig users to interact.

The presentations from this MRF meeting and previous meetings are available on the website [www.minipig-researchforum.org](http://www.minipig-researchforum.org). Minipig users can also share knowledge and experiences by joining the Minipig Research Forum LinkedIn Group.

# Catheter Workshop

**At this practical hands-on course you will try out different methods of catheterisation and you will have time to repeat the methods and thereby increase your skills.**

The specific course contents will be tailored to your exact needs and interests.

Examples of techniques are central venous catheters - either percutaneous (Seldinger technique) or surgically implanted with a subcutaneous port (VAP).

Furthermore, we will introduce you to the various types of materials and kits used for vascular access and discuss their pros and cons.

The course instructors are our laboratory technician and DVM.

We have scheduled three catheter workshops:

**6 March**

**30 April**

**25 June**

More catheter workshops can be scheduled.

For further information please contact us.

# Surgery course

**We provide regular training courses in anaesthesia and surgery of minipigs at our breeding facility in Dalmose, Denmark.**

The courses have a strong practical component with plenty of hands-on opportunities for you to gain experience by performing techniques yourself under close supervision of experienced tutors. When attending a surgery course you will be provided with instructive training which will add value not only to your daily work but also to the quality of science, welfare of the animals and research productivity.

The course instructors are Scientific Consultant Tony Webb and our DVM.

We have scheduled three catheter courses:

**15-16 May**

**4-5 September**

**4-5 December**

More surgery courses can be scheduled.

Please contact us to register or request further information.

# Catheters for Vascular Access and the Göttingen Minipig

Adrian Zeltner – az@minipigs.dk

*Ellegaard Göttingen Minipigs*

## Introduction

The main reason for using a venous catheter is to facilitate multiple blood sampling or intravenous dosing. It reduces stress to the animal, improves welfare, and reduces the number of personnel required.

This article will only cover catheters that are implanted by the Seldinger technique. There are some risks and challenges to this method; however, properly managed, it can be successfully applied after some training.

The purpose of this paper is to inform about material and methods that have been tested and used by Minipig users and at Ellegaard Göttingen Minipigs. Some users have sent in complete articles; others have contributed valuable data through personal communication with the author. As studies vary a lot in purpose and design we can by no means cover all possibilities for the use of catheters nor all types of material. Preferences are subjective and what works in one setting might not be appropriate in another. The idea is to give you an overview and inspiration, so you can build upon the experience of others.

## Summary

There are several types of catheters that could potentially be used with a Göttingen Minipig. A central venous catheter (CVC) is, by definition, a catheter whose tip resides in the central circulatory system. It is often inserted in close proximity to the heart. A PICC is a peripherally inserted central catheter and a Midline catheter is peripherally inserted without ending up in the central circulatory system.

There are various manufacturers of catheters that can be implanted using the Seldinger technique and each offers a multitude of models in different lengths, calibre, coatings, etc. They are monoluminal, biluminal or multiluminal and are almost all made of PU. Common to all is that they are designed for humans and not for pigs, so it can be a bit tricky to find the model that suits a particular study best.

These types of catheters are intended for short-term use; patency of the catheter can be anything between 3 and 28 days, depending on a multitude of factors.

In a biluminal catheter, one lumen can be used for dosing and the other for sampling or it can give an extra line when the other is occluded.

Loss of patency and infections are the main delayed complications whereas arterial puncture and impossibility to advance the guide wire could be complications during insertion.

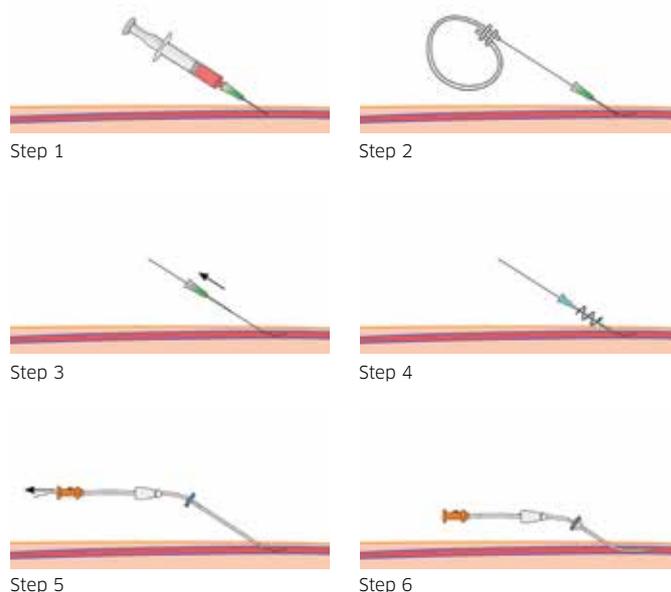
Catheterisation should be executed using aseptic procedures. It is done under general anaesthesia, using the Seldinger or modified Seldinger technique, the day before sampling.

## Seldinger Technique

The Seldinger technique is a medical procedure to obtain safe

access to blood vessels and other hollow organs. It is named after Dr Sven-Ivar Seldinger (1921–1998), a Swedish radiologist who introduced the procedure in 1953.

The desired vessel is punctured with a sharp hollow needle. A round-tipped guide wire is then advanced through the lumen of the needle into the vessel and the needle is subsequently withdrawn. A blunt cannula or dilator can now be passed over the guide wire to prepare the way for the catheter. After the dilator has been withdrawn, the catheter can now be passed over the guide wire and inserted into the vessel to the required length. After that the guide wire is withdrawn.



There are some modifications to the above method, like placing an over-the-needle catheter first and then feed the guide wire through it.

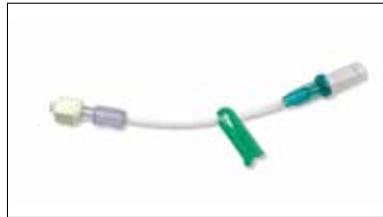
## Material

Traditionally larger Göttingen Minipigs have been catheterised because lifting and handling becomes more challenging with the increase of age and weight. Therefore large-calibre catheters were used, but as the procedures were applied in a broader spectrum of animal sizes and intentions, many different products came into use.

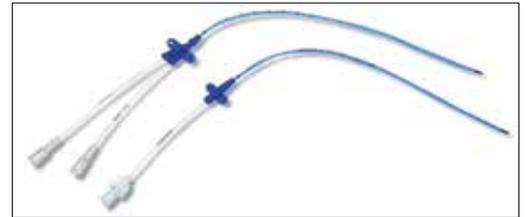
The following list gives an overview of catheters that have successfully been used in Göttingen Minipigs. It is based on user feedback, but is by no means complete. Most of them are monoluminal; some have an integrated extension, others do not. You will probably have to try out a few to find the one that fits your taste and purpose. The use of a needleless valve at the port has been proven to be very handy.



Catheter without extension



Extension with valve



Catheter with integrated extension

Manufacturer	Calibre Ga/Fr	Length, cm	Veins used	Reference
Cook Medical	20/3	8	Jugularis/Saphena	C-PUM-301J
Edwards Lifesciences	20/3	13	Jugularis/Saphena	M1 20130 HS
Arrow	14/7	16	Jugularis	ES 04706
	18/4	20	Saphena	ES 04218
Braun	20/3	12	Jugularis/Saphena	AK 04150 E-S
	14/7	15	Jugularis	Certofix mono S415
	18/4	15	Jugularis	Certofix mono S215
	18/4	20	Saphena	Certofix mono S220
	16/5	30	Jugularis	Certofix mono V330
	16/5	32	Jugularis (peel away intro.)	Cavafix certo 335
	13/7 (biluminal 16/16g)	30	Jugularis	Certofix duo V730
BD	20/3	15/20	Saphena/Auricularis	Careflow 681639
Mila	Mila International has a range of interesting catheters and accessories			milainternational.com

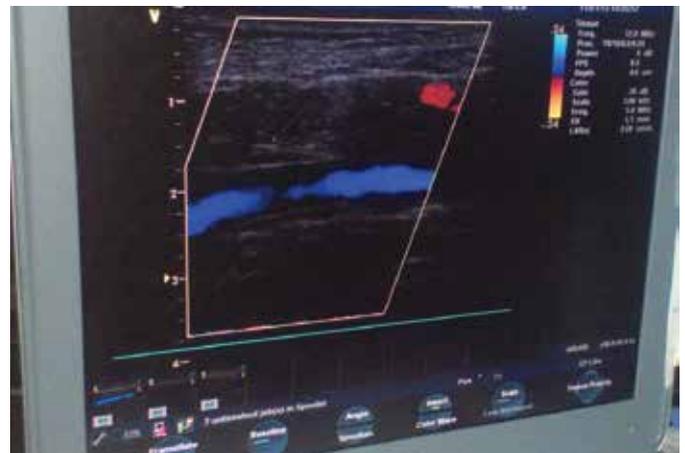
## Sites

### Neck - CVC

The most common site is the neck where the external jugular vein or the junction of external/internal jugular vein is catheterised. The challenging aspect of this site is that it is a blind procedure to penetrate the vessels as they are deep in the tissue and are not visible. Ultrasound guidance could be used to facilitate this step. It requires a bit of training with this relatively expensive equipment to successfully take advantage of this method but has the advantage that the correct vessel is punctured with the least damage to other tissue.



Transponder and needle at step1



Monitor showing vessel and needle(arrow)

Bandaging the neck can be a bit of a challenge as well due to the anatomy of the pig, but the port of the catheter can be embedded in it so it can be easily taken out at the time of sampling. Depending on the temperament of the respective animal, sampling can take place with the animal unrestrained or placed in a sling. Re-implantation of catheters at the same site has been possible in some cases, but other people reported that they were not successful the second time.

### Hind leg – Midline catheter

The saphenous vein is usually prominent and visible in the Göttingen Minipig. The leg can easily be bandaged and, as pigs

are not very athletic, it is impossible for them to interfere with their own bandage. A midline catheter can easily be placed at that site. The preferred technique is to gain venous access by placing an over-the-needle catheter first and then feeding the guide wire through this temporary catheter. In an initial study, 18g 20 cm catheters have successfully been used; the position of the tip was found to be in the area of the hip joint in a 12 kg Minipig. Re-implantation at the same site however proved to be impossible due to changes in the tissue around the place of insertion. Further studies are needed with small-calibre catheters to see if it has the same effect on the vein and surrounding tissue. The use of longer catheters, so that the tip rests in the caudal vena cava, could also be considered.

the other sites, as some narrow corners have to be navigated during insertion. In a 15-kg minipig, the tip of a 15 cm catheter rests in the middle of the external jugular vein and with a 20 cm catheter it is just before the right atrium.



Catheter placed in saphenous vein



### Conclusion

The placement of catheters in the Minipig is a valid option in the sense of the three Rs; however, each case has to be considered carefully in regards to benefits achieved. The minimal invasive techniques described here can easily be trained but some challenges remain. The main limitation is the duration of patency and re-implantation issues. The preferred site for implantation is the neck and it has been proven to be effective in practice. The hind leg has some potential but more work is needed to establish a method that is practically viable. There is also room for refinement in regards to the type of catheter suitable for Minipigs; more work is needed also in this area.

### Comments

If you have some experience with catheters you would like to share with others, you are welcome to write an article for our next newsletter or contact the author so it can be included in the next update or in our Guide for implantation of catheters using the Seldinger technique in the Göttingen Minipig. This guide is available from Ellegaard if you are interested in learning more about the subject. You are also welcome to join one of our catheter workshops where you can train the procedures in practice.

### Acknowledgements

I would like to thank the numerous dedicated researchers who contributed to this article. Although most of them cannot be named for confidentiality reasons; the others are:

- Dr Maike Heimann, Tierschutzbeauftragte ETH Zürich, Switzerland
- Kristine Eraker Aasland Hansen, DVM, Norwegian School of Veterinary Science, Norway
- Christophe Bory, Ricerca Biosciences SAS, Lyon, France
- Trine Pagh Ludvigsen, PhD student at the LIFEPHARM Center, Denmark
- Céline Monzali, DVM, Amatsi Avogadro, Toulouse, France



Minipig with bandaged catheter in pen

### Ear - Midline catheter

Some attempts have been made to place a catheter in the auricular vein. It can be successful if the Minipigs are large and the vein well developed. A 20-kg or lighter minipig is the only option here and even then the lumen of the vein is completely occupied by the catheter severely reducing the circulation through that vessel. That will result in changes of the tissue and re-implantation is definitely out of the question. The small size of the vein makes it a difficult technique as well, compared to

# Evaluation of blood sampling in the Göttingen Minipig through a venous catheter implanted according to the Seldinger technique

Bory, Christophe; Chalencon, Estelle; Baudet, Stéphane

Ricerca Biosciences SAS, Lyon, France

## Introduction:

Blood collection in the Göttingen minipig in toxicology studies is routinely performed from the jugular vein of conscious and restrained animals, generally by direct puncture of the vessel with a needle-and-syringe assembly. This technique generally requires significant human resources; even more staff may be needed when several blood draws are to be performed in a short period of time. One way to circumvent these repetitive blood draws over a short time period is to implant a chronic indwelling venous catheter for the sole purpose of blood collection. Although we initially contemplated this approach, surgery and maintenance of catheter patency were considered too resource-consuming. Instead, we investigated the feasibility of acutely implanting an indwelling catheter in the jugular vein using the Seldinger technique.

## Material and Methods

### Animals and Study Design

In a first test, a 7-day pharmacokinetic study was conducted in six Göttingen minipigs weighing 6 to 11 kg. Animals were dosed every day and blood was collected before dosing, and 0.5, 1, 3, 7 and 24 hours after dosing, on days 0, 4 and 7. Catheters (Arrow ES-04150 20 g 12 cm) were implanted once for the whole study, one day before the start of dosing and a necropsy was performed after the last blood draw, for macroscopic evaluation of the veins and heart. In a second test, a 5-week study was conducted in six Göttingen minipigs weighing 5 to 8 kg. Blood was collected before dosing, and 0.5, 1, 4, and 24 hours after dosing, on days 0, 9 (week 2) and 30 (week 5). Catheters (Arrow ES-04150 20 g 12 cm) were implanted one day before the intended blood collection day, and a necropsy was performed after the last blood draw, for macroscopic and microscopic evaluation of the veins and heart.

### Catheter Implantation using the Seldinger Technique

Minipigs were premedicated and anaesthetised with medetomidine, ketamine and butorphanol. They were then placed in dorsal recumbency, and the neck region was shaved and thoroughly disinfected with povidone iodine and ethyl alcohol. Catheter implantation was performed with material dedicated to the Seldinger technique. A specific needle was first introduced into a jugular vein, to assess venous return. A metallic guide was then advanced inside the vein through the needle and a dilator was subsequently introduced along the metallic guide through the skin and subcutaneous tissues. This manoeuvre was intended to facilitate the insertion of the catheter through the skin and adjacent subcutaneous tissues. The dilator was removed and the saline-prefilled catheter was finally introduced into the vein along the metallic guide, which was eventually removed. The catheter was sutured to the skin with two stitches. Once securely in place, this central catheter allowed repeated collection of venous blood from Göttingen minipigs placed in a sling.

## Results

### Short-Term Pharmacokinetic Study

One out of six animals was found disimplanted on day 0, after the first blood collection. In order to respect the sampling schedule, all remaining blood draws from this animal were acutely performed with the needle and syringe method. We were not successful in re-implanting this animal with the Seldinger technique in the same jugular vein, possibly because the several acute blood draws might have caused tissue damage and prevented further catheter insertion.

Between days 4 and 7, one catheter was accidentally disimplanted by a technician during animal handling. This animal was successfully re-equipped with another catheter using the Seldinger technique.

In five central catheter-equipped animals, all blood collections were readily performed and the quality of the samples was good (no coagulated samples, target volumes always achieved). The mini-pigs were much quieter when placed in a sling than when manually handled. Macroscopic examination of successfully implanted minipigs did not reveal any adverse findings considered related to catheter placement.

### Five-Week Study

The insertion of the catheter in the jugular vein (right vein before sampling on day 0, and left vein before sampling on day 9) was successful in all animals. Nevertheless, insertion in the right jugular vein on the day before day 30 was very difficult, as shown by the inability to insert the metallic guide. This caveat was considered related to inflammatory remodelling of tissues after the first catheter insertion. In the present study, acute or chronic thrombus formation, associated with partial occlusion of the lumen, occurred in the jugular vein. Sporadic thrombosis occurred in the cranial vena cava as a consequence of the thrombus formation in the jugular veins. Considerable thrombus formation occurred in the right atrium or ventricle which was considered to be the consequence of vascular changes in jugular veins and/or cranial vena cava. When prominent, the thrombus formation especially in the heart may represent a serious life-threatening condition.

## Conclusion

A central venous catheter could be readily placed in the jugular vein of Göttingen minipigs via the Seldinger technique, with minimal surgery. Several blood draws could be successfully performed during a single day, with minimal stress for the minipigs placed in a sling. This technique did not appear to be as well-suited when several blood collections were performed in the same vein several weeks apart, probably because of significant tissue remodelling and thrombosis. In our hands, central venous catheter implantation with the Seldinger technique appears better adapted to repeated blood collections, for instance during short-term toxicokinetic studies (about one week in duration).

Fig. 1 : Blood collection in a Seldinger-catheterised minipig in a sling.



# Refinement: Implantation of Central Venous Catheter in Minipigs

## The experiment:

In March 2012 our department conducted a toxin experiment on minipigs. We bought 4 minipigs from Ellegaard Göttingen Minipigs. The minipigs were acclimatised for 4 weeks during which time the minipigs were housed in a group. The day before the experiment started, the pen was divided into 4 individual pens and we implanted central venous catheters in all 4 minipigs. The day of the experiment all 4 minipigs were fed the toxin in the feed, 3 different toxins plus 1 control. Blood samples were collected every other hour and all faeces were also collected. After 24 hours the minipigs were sacrificed and necropsied.



## Refinement:

Our department works for animal welfare and refinement and we were able to do so in this experiment. We conducted a pilot study for this experiment, with one minipig, in 2011, and with our experiences from the pilot study and tips from several sources we were able to refine the implantation of the central venous catheter for the main experiment.

First, I would like to thank Ellegaard Göttingen Minipigs for all their help and service. They helped us a lot with this experiment. We used Ellegaard's protocol for implantation of the central venous catheter. And they helped us refine the bandaging that we used to fixate the catheters.

They recommended the Optiplaste Duct tape which is more flexible and softer than the normal duct tape we had used during the pilot study, which resulted in oedemas in the minipig jaw area. When using the Optiplaste Duct tape we had no oedemas.



We also had help from our anaesthesia department to refine the anaesthetic used for the implantation of the central venous catheter. During the implantation of the central venous catheter during the pilot study, we used the Zoletil mix, but after some concern from the anaesthesia department about the minipigs' venous pressure, especially seeing that the procedure of implantation of the central venous catheter requires good venous pressure, we decided to use a combination of medetomidine, butorphanol and propofol. This combination can also be reversed with atipamezol after the procedure is done. Using this anaesthesia we had no problems with the venous pressure and since we reversed the anaesthesia after the procedure was done, we reduced the recovery time considerably.

During the pilot study the minipig turned blue during the procedure, as it was laying on its back under anaesthesia for a long period of time. For the main experiment, we gave the minipigs oxygen via a mask from an oxygen concentrator. When using the oxygen concentrator, none of the minipigs turned blue.

In the pilot study we used the Braun Certofix Mono S 415 (6fr/15 cm) central venous catheter, but it was too long for the small size of minipig (10 kg) we were using and we had problems with the catheter sliding out and bending. After consulting with a colleague who uses the same procedure, we decided to use the Cook Medical C-PUM-301J (3fr/8 cm) catheter. This catheter is so short that you can insert it totally and imbed the end before suturing. A 10-cm Luer Lock BD Connecta extension with a three-way stop lock was then attached; the port rested in the bandage and could be exposed for sampling. The catheter was flushed with NaCl with Heparin between blood sampling. When using the Cook Medical catheter we had no problems with sliding or bending of the catheter. All the blood samplings were successful.

I hope these tips will be informative for others who use this procedure and that this will result in refinement of your procedures and better welfare for the minipigs.

Kristine Hansen (mail: [kristine.hansen@nvh.no](mailto:kristine.hansen@nvh.no))  
Clinic Veterinarian  
Section of Experimental Biomedicine  
Norwegian School of Veterinary Science

# Prolonged Anaesthesia in Minipigs

R. Eddie Clutton, Frances Reed, Michael Eddleston\* and Elspeth Hulse\*

Royal (Dick) School of Veterinary Studies, The University of Edinburgh, United Kingdom

\* Clinical Pharmacology Unit, University/BHF Centre for Cardiovascular Science, The University of Edinburgh, United Kingdom

Prolonged anaesthesia may be required in minipigs undergoing terminal toxicological, pharmacological or microbiological studies in which the onset of action of test substances or infective agents is slow. It is also necessary in studies using pigs as models for the human intensive care patient. Being able to maintain anaesthesia for as long as possible is desirable because it maximizes the data volume that may be collected from individual pigs. Maintaining a stable physiological state minimizes data variability and increases study power. Both will contribute to *reducing* the number of animals required in a given project. Maintaining physiological variables within appropriate limits is particularly important for recovery experiments in which deviations from normality may compromise convalescence rate and welfare and so undermine attempts at *refinement*. The principles of reduction and refinement underpin the justification for the ongoing use of animals in biomedical research (Russell and Burch 1959).

A biomedical literature review reveals considerable variation in the definition of the term “prolonged”; see Table 1. However, describing “prolonged” in units of time ignores the term’s implicit link with increased anaesthetic risk. Risk arises from the experiment itself, and from the skills and experience of those responsible for the anaesthetic. As the former is a “fixed effect” it is appropriate that any definition of “prolonged” should embody a sense of challenge to the latter. One option is to define a “prolonged anaesthetic” as one whose duration is expected or planned to exceed the anaesthetists’ previous experience by a pre-determined factor.

Previous work (table 1) has focused on the anaesthetic drugs used to produce prolonged anaesthesia. However, diligent attention to non-pharmacological factors (Table 2) is important in maintaining stable conditions and preventing unexpected

complications in prolonged procedures. Such attention becomes even more important in survival experiments where the goals are to minimize postoperative morbidities while restoring normal function as rapidly as is consistent with the animal’s welfare. The following article is based on personal experiences and concentrates on the non-pharmacological aspects of prolonged anaesthesia in minipigs.

Table 2. Factors requiring attention in prolonged anaesthetics in pigs

Subject selection: pre-operative examination
Pre-operative preparation
Drugs / technique selection
Depth of anaesthesia monitoring
Venous access and surgical cleanliness
Airway management
Anaesthetic breathing system selection
Inspired O2 levels
Ventilation mode
Temperature management
Fluid and blood loss management
Urinary bladder distension
Glucose and electrolyte management
Body position
Eye protection
Oral hygiene
Monitoring & tedium - Maintaining vigilance

## 1) Subject selection: pre-operative examination

The potential for problems arising during and after prolonged anaesthetics (in the case of survival procedures) is reduced by ensuring subjects are in optimum health. The animal’s recent medical history, including body mass changes, coupled with a physical examination establishing health is a minimum require-

Table 1. Publications on pig anaesthesia incorporating the term “prolonged” in the title.

Citation	Pigs				Duration (hours)
	n	breed	mass (kg)	sex	
Cummings et al (1972)	67	Yorkshire, Landrace, Hampshire, Duroc crosses	165 - 323	♀	5 - 13
Holmes et al (1990)	8	Landrace crosses	15 - 20	♀	7 - 9
Mutch et al (2000)	20	?	20 - 30	?	7
Nosser (2003)	20	Pietrain X Hampshire	36 - 50	♀	7
Kilic & Erhardt (2004)	9	?	30 (approx)	?	4
Eddleston et al (2012)	> 100	Göttingen minipigs	15 - 30 kg	♂♀	24 - 48

?: undisclosed



ment. Haematological and biochemical testing may be justified at this stage in providing baseline values that may prove useful in monitoring recovery and convalescence, rather than indicators of health.

## 2) Pre-operative preparation

No pre-operative preparation is required in healthy pigs beyond some degree of food and water deprivation. Water should be withheld once pre-anaesthetic medication has been given. However, the appropriate degree of food deprivation required is controversial. In our laboratory, pigs are fed 500 g per pig per day, once per day in the morning. The pellets are spread on the floor so the pigs take a few hours to find them amongst the straw. They are not fed on the morning of surgery until the animals scheduled for anaesthesia are removed. However, some pigs eat the straw upon which they bedded when they appear to become hungry in the early morning. This may be related to what appears to be the initial stages of necrotizing colitis seen at necropsy in a proportion of pigs after 24 - 48 hours of anaesthesia, and which appear to be associated with a full large bowel. Whilst some pigs spontaneously defecate during anaesthetics, it is possible that anaesthetic drugs and/or surgical stimulation exert constipative effects in others. That this may lead to impaction and associated problems in survival experiments prompts the consideration of aggressive gastro-intestinal evacuation measures, such as purgatives, enemas and extensive fasting before prolonged procedures. However, such measures are probably stressful and may adversely affect glucose homeostasis.

## 3) Drugs / technique selection

The anaesthetics chosen must produce experimental conditions whilst having minimal - or at least predictable effects on study variables. Side-effects should be manageable. In recovery experiments, anaesthetics must have non-cumulative properties. While volatile agents and total intravenous techniques have distinct advantages and disadvantages, modern practice favours a hybrid technique in which anaesthesia is produced using low inspired concentrations of insoluble volatile anaesthetics like sevoflurane or isoflurane, whilst analgesia is provided by constant rate infusions (CRIs) of short-acting opioids like fentanyl or alfentanil.

## 4) Depth of anaesthesia monitoring

Monitoring and controlling the depth of anaesthesia is necessary if rapid recoveries are to occur because these depend on the administration of the lowest doses of anaesthetic consistent with the production of experimental conditions. Furthermore, a constant depth of anaesthesia is often necessary because changes in anaesthetic depth resulting from changes in anaesthetic dose will invariably be associated with unstable physiological conditions. Constant dosing is possible with injectable techniques, e.g., CRIs and target-controlled infusions (TCI), or when volatile agents are delivered to produce constant end-tidal concentrations. Unfortunately, constant dosing techniques do not assure stable levels of anaesthesia, because other factors affect depth, the most important of which is probably body temperature. Consequently, in some experiments it may be more appropriate to produce a constant level of central nervous depression rather

than the constant administration of anaesthetic. Unfortunately, signs of anaesthetic depth in pigs are rarely "scalable": corneal reflexes are either present or absent, and do not become sluggish as anaesthesia deepens. Jaw tone is an exception to this generalisation. Whilst evidence for the effectiveness of electroencephalographic-based methods of anaesthetic depth monitoring, e.g., the bi-spectral index (BIS) has been difficult to establish experimentally, we have found BIS monitoring to be near-invaluable in prolonged minipig anaesthetics (figure 1).



Figure 1

## 5) Venous access

Venous access may be established in the marginal auricular vein of the sedated pig for the purpose of inducing anaesthesia with intravenous agents. In prolonged procedures, it is necessary to ensure higher levels of surgical cleanliness when cannulating blood vessels and performing other "routine" instrumenting procedures, because infection becomes more likely the longer implants are in place. There is a greater justification for prophylactic antibiotics.

## 6) Airway management

The airway must be protected when anaesthetics eliminate protective airway reflexes. The intubation of a tracheal stoma provides a secure airway in acute procedures although oro-tracheal tubes are used when recovery is planned. Both cuffed endotracheal and tracheostomy tubes left *in situ* for prolonged periods may lead to traumatic and/or ischaemic tracheitis. The latter is more likely when cuffs are over-inflated. The cuff should be inflated to a pressure just in excess of the highest pressure required to produce lung inflation when positive pressure is imposed (see below). In prolonged operations, the cuff should be periodically deflated and re-inflated after the tube is repositioned 1 - 2 cms craniad or caudad. Sterile (new) endotracheal tubes should be used in pigs undergoing prolonged anaesthetics.

The prolonged inspiration of anaesthetics carried in dry medical gases (and the expiration of gases saturated with water vapour)

leads to dehydration of the tracheobronchial mucosa which paralyzes the mucociliary carpet and viscifies airway secretions. Accumulated airway secretions may occlude distal airways and by increasing intrapulmonary shunt lower blood  $O_2$  tensions. Consequently, thought may be given to the intraoperative humidification of inspired gases, and in survival cases, performing endobronchial suction before tracheal extubation, providing post-operative expectorants and, or mucolytics, humidified air and thoracic percussion. The post-operative use of antitussive drugs must be considered as these will retard the expectoration of airway debris.

The prolonged loss of water vapour from the tracheobronchial tree also leads to considerable heat loss. This may be conserved with suitable breathing systems or the use of heat and moisture exchangers (HMEs) interposed between the breathing system and the endotracheal tube connector (figure 2).



Figure 2

### 7) Anaesthetic breathing systems

The choice of anaesthetic breathing system is critically important when pigs are expected to breathe spontaneously during the procedure, but selection is not straightforward. Non-rebreathing systems, e.g., the Bain system, offer little resistance to breathing and so minimize the work of breathing over extended periods. However, they do not conserve expired water vapour and their capacity to warm inspired breath is questionable. Moreover, the high gas flows required may prove expensive. Rebreathing systems like the circle and “to-and-fro” are regarded as high resistance systems but are more effective at conserving respiratory heat and moisture. The choice is academic when the lungs are mechanically ventilated.

### 8) Inspired $O_2$ levels

Prolonged exposure to high concentrations of inspired  $O_2$  adversely affects respiratory function in several ways. However, information on safe exposure levels in minipigs does not appear to exist. In dogs it is recommended that exposure to 100%  $O_2$  is limited to no more than 24 hours, or to 48 hours when 60%  $O_2$  mixtures are breathed. In prolonged procedures,  $O_2$  concentrations from the anaesthetic machine may be reduced by dilution with medical air. Unfortunately, many veterinary anaesthetic machines do not possess the capacity to deliver air,

but information on simple modifications is available (Clutton et al 2012)

### 9) Ventilation mode

Allowing pigs to breathe spontaneously throughout a prolonged anaesthetic confers some advantages: respiratory pattern and rate are useful indicators of anaesthetic depth and the approach is straightforward – no equipment beyond a suitable breathing system is required. However, the work of breathing is provided entirely by the animal; respiratory “fatigue” is possible in prolonged anaesthetics, particularly if resistance to breathing is increased. During spontaneous breathing minute for minute changes in the inspired gas volume will result in the uptake of different levels of inhaled anaesthetic and contribute to unstable anaesthetic depth. Spontaneously breathing animals will hypoventilate (if adequately anaesthetized) which will elevate blood  $CO_2$  levels and lower pH. Furthermore, during spontaneous breathing dependent lung tends to collapse if not periodically inflated. Areas of diffuse atelectatic lung fails to oxygenate blood and by increasing venous admixture will lower blood  $O_2$  levels. The progressive collapse of dependent lung tissue is retarded by “sighing” – the imposition of a supra-normal lung inflations at 3 – 5 minute intervals. However, diffuse pulmonary microatelectasis is best prevented by controlling ventilation throughout anaesthesia.

By imposing constant gas volumes at a fixed frequency, controlled ventilation maintains stable blood gas values & pH and stabilizes “depth”. It also preserves metabolic energy. However, the use of respiratory pattern as a “depth” indicator is lost and there are adverse physiological effects: a reduction in cardiac output and accelerated heat loss being the most important. Prolonged controlled ventilation is synonymous with mechanical ventilation and the use of mechanical ventilators, because the other option, manual inflation, is infeasible in operation lasting more than several hours. The prolonged inappropriate use of mechanical ventilators is associated with ventilator associated lung injury (VALI).

Ventilator associated lung injury results from excessive pressures (barotrauma), excessive distending volumes (volutrauma), alveolar damage resulting from transient and repeated closure and reopening of alveoli during the respiratory cycle (atelectrotrauma) and biotrauma, in which the altered magnitude and pattern of lung stretch changes gene expression and cellular metabolism in a way that produces an overwhelming inflammatory response – even in the absence of structural damage. The latter phenomena, known as systemic inflammatory response syndrome (SIRS) has been investigated in the porcine model and can be fatal within 12 – 24 hours of a relatively brief period (8 – 12 hours) of injudicious (excessive tidal volumes over prolonged periods) lung ventilation. The risk of SIRS is reduced by using the ARDSnet protocol, a lung ventilation pattern established by syndicated United States health agencies committed to the treatment of acute inflammatory lung disease. The ASARDSnet protocol, which is detailed online (<http://www.ardsnet.org/system/files/Ventilator%20Protocol%20Card.pdf>) involves the delivery of low tidal volumes ( $6 - 8 \text{ mL kg}^{-1}$ ) at relatively high respiratory rates. Unfortunately, the complex ventilatory



patterns required by the ARDS protocol requires intensive care, rather than anaesthetic ventilators. The former are more complicated, expensive and less available than the latter (figure 3).



Figure 3

### 10) Temperature management

Anaesthetized pigs inevitably lose heat because anaesthetics and surgery promote heat loss; while compensatory behavioural and physiological responses to hypothermia are impaired by anaesthetics. In the absence of interventions, e.g., externally applied heat, temperature loss is a function of time and the ambient temperature. Hypothermia is undesirable in all operations because it affects numerous physiological variables and so contributes to variability in collected data. It contributes to central nervous depression and so augments the effects of anaesthetics; it may contribute to relative overdose and prolonged recoveries. It reduces ventilation and cardiac output, which will affect the uptake and distribution of inhaled anaesthetics and retard their elimination. In terms of tissue oxygenation, its adverse effects on cardiac contractility are augmented by increasing blood viscosity and a left-shift in the oxyhaemoglobin dissociation curve. At core temperatures of approximately 28°C ventricular fibrillation is near-inevitable.

In recovery procedures, the hypothermic mammal whose capacity for thermoregulation returns with recovery from anaesthesia mounts a hypermetabolic heat generating response that in humans is unpleasant, and which may exhaust limited glucose and O<sub>2</sub> reserves.

Hypothermia is more appropriately prevented than treated. Numerous strategies are feasible including the use of high ambient



Figure 4

(laboratory) temperatures or creating warm “local” conditions (bubble wrap insulation with hot air blankets and/or heater pads; see figure 4). During invasive procedures, irrigation fluids should be warmed to 37 - 38°C. The anaesthetist minimizes heat loss by avoiding high doses of (vasodilating) α<sub>1</sub> antagonist drugs, e.g., azaperone, using positive pressure ventilation judiciously, favouring “low-low” re-breathing systems and using HMEs.

In recovery experiments “cold” pigs which are slow to recover must be given O<sub>2</sub> by mask whenever shivering is present. Core temperature can be raised in numerous ways but over-enthusiastic warming must be avoided as the risk of burns is greater in animals with impaired thermoregulatory reflexes and cutaneous blood flow. Recovering animals should be thoroughly dried (if wet) with towels and hair dryers. Topical heat may then be judiciously applied using 40 watt light-bulbs, radiant infra-red lamps or insulated hot-water bottles. Topical internal heating methods, i.e., peritoneal, pleural, gastric or rectal lavage, are highly effective but each has specific disadvantages, e.g., peritoneal and pleural lavage may exert significant dialysing effects. Haemolysis will occur if intravenous fluids are warmed > 40°C.

### 11) Fluid and blood loss management

Fluid balance is extremely important in prolonged anaesthetics. If the simple principle that fluids lost must be replaced on a “mL-for-mL” and “like-for-like” basis is ignored then either fluid overload or hypovolaemia will ensue. Overinfusion of crystalloid solutions will result in 3rd space losses, including pulmonary oedema. The excessive use of colloids will result in systemic and pulmonary hypertension, and ultimately ventricular failure. The net loss of circulating blood volume over time will result in signs of hypovolaemic shock, e.g., arterial hypotension, oliguria and depressed mentation.

Water and electrolyte losses are inevitable in the anaesthetized pig because urine production *should* continue while free water is lost by evaporation in the lung. These losses are compounded by surgical blood loss. The latter is easily quantified:

mLs blood lost = (mass [in g] of N bloody swabs - mass of N dry swabs) / 1.3

The volume of blood lost to surgical suction in suction jars can also be quantified:

$\text{mLs blood lost} = (\text{PCV in suction jar} / \text{pig PCV}) \times \text{mLs blood in suction jar}$ .

Water and electrolyte losses (and crystalloid requirements) are more difficult to quantify although empirically, infusing poly-electrolyte solutions at 5 - 15 ml kg<sup>-1</sup> hour<sup>-1</sup> during minor - major operations usually maintains cardiac preload and sustains urine output without obvious adverse effect.

### 12) Urinary bladder distension

Continued urine production - which is desirable - will result in progressive urinary bladder distension and a rise in intravesicular pressure - which is not desirable: excessive pressures will cause post-renal failure and cystitis. In recovery experiments full bladders cause extreme discomfort. Neither minipig boars nor sows seem able to void full bladders passively under anaesthesia and so some method of urine evacuation is required. The digital introduction of a urethral catheter is occasionally - but not always achievable *per vaginam* in sows. In prolonged anaesthetics in minipigs of both sexes, a surgical cystotomy is recommended. This must be performed under sterile surgical conditions as ascending catheter associated urinary tract infections are not uncommon.

### 13) Glucose

During prolonged anaesthetics blood glucose levels frequently fall from 2.7 - 4.3 mmol L<sup>-1</sup> to < 2.0 mmol L<sup>-1</sup> over 18 or more hours. This progression can be retarded by infusing 4.3% dextrose 0.18% saline solutions at 5 - 15 ml kg<sup>-1</sup> hour<sup>-1</sup>. When blood glucose falls < 2.0 mmol L<sup>-1</sup>, dextrose (50%) injections are recommended at 0.5 mL kg<sup>-1</sup>.

### 14) Body position

Intra-operative pig position should be optimized for surgery or experiment but without adversely affecting blood flow and ventilation. Ties must be used carefully (figure 5); when overtight, they exert a tourniquet-like effect and cause tissue and nerve damage. The imposition of abnormal positions for prolonged periods may also lead to nerve and/or muscle damage.



Figure 5

When arthritis is present, severe discomfort can be expected after prolonged joint flexion in (normally) intolerable positions. This is extremely undesirable in animals involved in recovery experiments. Periodic position changes (if feasible) and some simple physiotherapeutic manipulations may be considered in pigs recovering from prolonged experiments.

### 15) Eye protection

Prolonged anaesthetics in which the eyes remain partly or fully open will lead to corneal desiccation, post-operative discomfort and possible corneal damage. The ocular surface must be kept moistened and/or protected by taping or bandaging the eyes shut, or applying artificial tears, e.g., "Lacrilube", at frequent intervals.

### 16) Oral hygiene

The proliferation of bacteria in the oral cavity over time contributes to ventilator-associated pneumonias in humans undergoing intensive care. In pig models, cleaning the teeth three times daily and applying 0.2% chlorhexidine gel afterwards may prevent similar problems, and in any case is justified as it contributes to a "truer" model.

### 17) Monitoring & tedium - Maintaining vigilance

Prolonged anaesthetics in our laboratory involves individual shifts of 8 hours. At least two people are present at all times, but especially at night. Food and drink are available immediately outwith the laboratory area. When data loggers are used, continued surveillance and care of the unconscious pig can be promoted by designing studies that demand periodic attention to the animal and the manual recording of data.

### Conclusion

Prolonged anaesthesia in pigs for biomedical research is challenging and success depends as much on critical attention being paid to non-pharmacological factors as it does to the anaesthetic technique. Attention to these factors becomes critically important in recovery experiments because it will affect the comfort and post-operative welfare of the animal.

### References

- Clutton RE, Schoeffmann G, Chesnil M, Gregson R, Reed R, Lawson H, Eddleston M. Reducing the Oxygen Concentration of Gases Delivered from Anaesthetic Machines Unadapted For Medical Air. *Veterinary Record* (2011) doi: 10.1136/vr.d4928
- Russell, W.M.S. and Burch, R.L., *The Principles of Humane Experimental Technique*. Methuen, London, 1959.

# Experiences from using the Göttingen Minipig as a model of human genital chlamydia infection

Karin Erneholm, DVM, PhD student (kaer@sund.ku.dk)

Sarah Bøje, MSc, PhD student (sarahboeje@sund.ku.dk)

Jørgen Agerholm, DVM, PhD, Professor (jager@sund.ku.dk)

*Veterinary Reproduction and Obstetrics, Department of Large Animal Sciences,  
Faculty of Health and Medical Sciences, University of Copenhagen*

**Collaboration between the University of Copenhagen and Statens Serum Institut has enabled two PhD students to evaluate the use of a porcine model in the field of genital chlamydia research. The projects focus on primary genital infections, but also on evaluation of the protective properties of vaccine candidates against subsequent infection. Here, we give a background to the projects and share some practical experiences from the studies.**

## Background

The Chlamydiaceae family covers a wide variety of subspecies and serovars. The bacteria are mainly host specific, but cross infections occur. Some species have zoonotic potential, some of which may have serious adverse effects on humans. For example, *Chlamydia avium* causes the pneumonia “ornithosis”, and human abortion can be caused by *Chlamydia abortus*, spreading from sheep.

The most well-known human chlamydia strains in the western world are those associated with genital chlamydia infection (the disease known as “chlamydia”). Chlamydia has a major impact on society, being the most common sexually transmitted bacterial disease. A genital chlamydia infection may cause infertility, ectopic pregnancies and long-term pelvic pain in women previously exposed to the infection. The infection has an asymptomatic course in up to 75% of the cases. Chlamydia is treatable by antibiotics, but since so many cases remain asymptomatic, controlling the infection and preventing complications by antibiotics is challenging. A vaccine would therefore be very beneficial.

A research group at Statens Serum Institut (SSI), Copenhagen, is currently developing a vaccine against chlamydia. Vaccine candidates are mainly screened using the murine model, but a need for testing potential vaccine candidates in an animal model with physiology closer to humans than mice made us consider using the pig for this purpose.

There were different reasons for us to choose the minipig. Since we wanted sexually mature animals, the smaller size of

the minipig compared to the conventional pig is of course of a great advantage. Also, the Ellegaard Göttingen Minipig was declared free of porcine chlamydia and we would not risk a cross reaction of *Chlamydia suis*, which is prevalent in conventional pig herds.

## Where are we now?

Since the project started in 2010, we have performed infection and immunisation studies. During this period, our methods have been optimised, both in the laboratory, but also in terms of handling and sampling. Here, we will share some of our experiences with minipig handling and sampling used for a genital tract infection study.

## Synchronising the oestrus cycle

The female pig has an oestrus cycle of about 21 days, and during this period different hormone levels influence both the physical appearance of the genital tract, as well as the local mucosal immune response. These factors can of course have an impact on the responsiveness of an acquired experimental infection. Moreover, one must be aware of the properties of the infectious agent, since microorganisms may be more infectious under oestrogen influence compared to progesterone influence (or the opposite!). It is therefore important to be able to synchronise the oestrus cycle of gilts to have them at the same hormone level at the time of infection.

We have good experience using the progestin “Regumate equine<sup>®</sup>” (altregonest) to synchronise the pigs in our studies.<sup>(1)</sup> To ensure the use of only sexually mature pigs, we used gilts around 6 months old, with a normal vulva size, and a previously observed oestrus. The pigs were fed 20 mg Regumate<sup>®</sup>/pig once a day for 18 days by pouring the Regumate<sup>®</sup> solution on top of the food. We did not notice any reluctance to eat the food completely despite the adding of Regumate<sup>®</sup> and therefore the pigs were likely to get the expected dose. The treatment has apparently not influenced the appetite and temper of the pigs. Sometimes we have noticed slightly more watery faeces. The vulvas were constantly without signs of increased blood flow (not swollen or red) during the treatment period.

1. Coffey, R. D., G. R. Parker, and K. M. Laurent. 1998 Manipulation of the estrous cycle in swine. Cooperative Extension Service, College of Agriculture, University of Kentucky, Lexington, Kentucky, USA. Available online : <http://www.uky.edu/Ag/AnimalSciences/pubs/asc152.pdf>.



Vaginal sampling  
(photo: Sarah Bøje)

Four days after the last day of treatment, the first animals started to show signs of oestrus, and within 8 days, all animals usually had shown signs of oestrus. The vulvas were swollen and red, and the pigs got very restless, compared to their general behaviour. In our experience, we had good results using a “teaser boar”, which was exposed to the gilts twice a day (2x15 min) through a gate made of iron tubes. The boar and the gilts were allowed to have nose-to-nose contact through the gate. In our opinion, using an old, “experienced” boar is the most efficient way to induce obvious signs of oestrus. For a smaller experiment, we also tried to do oestrus detection without a teaser boar. It worked out fine, even though the signs were not as obvious as when using a teaser.

One must remember that the duration of oestrus varies between individuals, and even though signs of oestrus might be obvious, it can be a good idea to combine this method with monitoring hormone fluctuations by blood sampling.

### Infection method

In human chlamydia, the disease typically starts with an infection of the vaginal and cervical epithelium, and from here, the infection ascends into the uterus. In order to introduce the bacterium to the right anatomical location, we aimed to inoculate the cervical epithelium. In the literature, insemination catheters have been used for genital infection studies using slaughter pigs. To our knowledge, there are no insemination catheters available for minipigs. We have used insemination catheters designed for dogs (Osiris) with good results.

Because of the length and complex internal winding structure of the porcine cervix, we could not insert the catheter deeper than to the very beginning of the cervix. We inoculated approximately 5 ml of bacterial suspension at this location. The pigs were anaesthetised during the procedure, and they lay for 20-30 minutes after the inoculation with the hind part slightly elevated. It is of course important to clean and disinfect the external genitalia to prevent the catheter from introducing environmental pathogenic agents into the deeper part of the vagina.

### Vaginal sampling

We sampled the vaginal surface at regular intervals after the inoculation. For our purpose, it was important to sample the innermost part of the vagina. The external genitalia were cleaned and disinfected to avoid contamination by bacteria from the outermost parts of the vulva and vestibulum. It was important to prevent induction of bacteria into the deeper parts of the vagina but also to avoid contamination of our samples. We find it best to sedate the gilts, and to use a vaginoscope designed for dogs. The vulva was cleaned gently with water and an ethanol solution before inserting the vaginoscope, which had been dipped in sterile paraffin oil beforehand. With help from the vaginoscope, we were able to insert the swab deep into the vagina without touching the vestibular and outermost parts of the vaginal wall.

If you have any questions or comments regarding our research and experimental techniques, please contact Professor Jørgen Agerholm, main supervisor on the PhD-projects: [jager@sund.ku.dk](mailto:jager@sund.ku.dk).

For more information regarding the chlamydia vaccine research at SSI, visit [www.ssi.dk/English](http://www.ssi.dk/English), or contact Frank Follmann, Head of Section, Chlamydia Vaccine Research: [frf@ssi.dk](mailto:frf@ssi.dk).

# Challenges to detecting the end of cardiac repolarisation in the Göttingen Minipig ECG

Florence Koeppel<sup>1</sup>, Michael Markert<sup>2</sup>, David Labarre<sup>1</sup>, Philippe Zitoun<sup>1</sup>

<sup>1</sup> NOTOCORD Systems, France, [www.notocord.com](http://www.notocord.com), [fkoeppel@notocord.com](mailto:fkoeppel@notocord.com)

<sup>2</sup> Boehringer Ingelheim Pharma GmbH & Co KG, Germany, [www.boehringer-ingelheim.com](http://www.boehringer-ingelheim.com), [michael.markert@boehringer-ingelheim.com](mailto:michael.markert@boehringer-ingelheim.com)

## Abstract

Minipigs exhibit characteristic and unique ECG features. In particular, the morphology of the T wave, which corresponds to cardiac repolarisation, is often observed to change on a beat-to-beat basis. This variability represents a challenge for automated ECG analysis, especially for defining the timing of the T-wave start and end. A new software application named ECG30s was developed specifically for automated analysis of ECGs with changing T-wave morphology. This study was designed to determine the accuracy of P, Q, R, S and T points positioning with this new software. To that end, a signal database was built with 17 ECG recordings, mainly from freely moving Göttingen minipigs. Positioning of fiducial points with the automated software was compared to positioning done manually. Results show a sensitivity of 80.3%, 85.5%, 97.3%, 89.1% and 80.7%, and a predictivity of 78.7%, 83.8%, 95.3%, 84.0% and 81.4% for P, Q, R, S and T points placement, respectively. In conclusion, ECG30s ensured consistent detection of T end even with changing T-wave morphology.

## Introduction

Minipigs have been used increasingly in cardiovascular safety and pharmacology studies (McAnulty, 1999, van der Laan, 2010) for a variety of reasons. Their small size compared to pigs make them relatively easy to handle in a laboratory setting. Their cardiovascular anatomy shares many similarities with human cardiovascular anatomy. Moreover, the use of minipigs in preclinical studies tends to have a greater overall acceptance with the public than the use of either dogs or primates.

The minipig ECG exhibits some characteristic features that need to be taken into account when developing a software tool for automated analysis (Kuwahara, 2004, Laursen, 2009, Stubhan, 2008). The S wave is usually two or three times higher than the R peak. The P-wave amplitude can be relatively small. The T wave can be either positive, negative or even bipolar. The QT-interval duration of the minipig is about 300 ms for a heart rate of 60 beats/min., i.e. 100 ms longer than for a dog. In addition, the T-wave morphology can change spontaneously on a beat-to-beat basis, and also over time in relation to the heart rate. For instance, T-wave amplitude is observed to increase when the heart rate increases. Of course these changes are also affected by the lead placement used to acquire the ECG. Figure 1 shows an example of T-wave morphology evolution in minipig.

Amongst all features, changes in T-wave morphology over time are the most challenging as far as automated analysis is

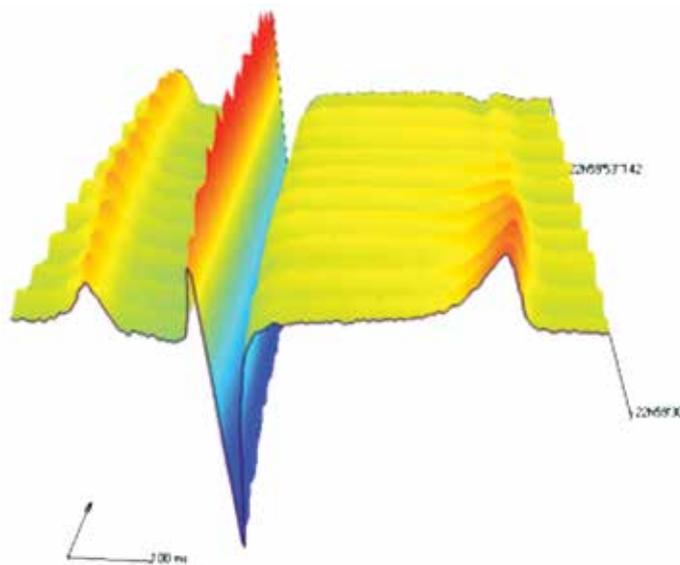


Figure 1. 3D view of a minipig ECG showing the evolution of T-wave morphology over 30 successive beats.

concerned, because they may occur on a beat-to-beat basis. One of the most popular approaches used by ECG analysis software relies on a pattern matching technique. However, it is not appropriate when ECG beat morphology varies on a beat-to-beat basis. To overcome this issue, NOTOCORD® developed a real-time ECG analyser named ECG30s, in the NOTOCORD-hem™ software platform (NOTOCORD Systems, France). The purpose of this paper is to characterise the performance of the ECG30s analyser on the positioning of the five standard fiducial points: P start, QRS start, R peak, QRS end and T end.

## 1. Methods

### 1.1. ECG signal database

ECG30s performance was characterised by its accuracy in positioning fiducial points in ECG signals. This was assessed by comparing automated analysis to manual analysis performed by experts. For this purpose, a validation database was built with 17 ECG signals. They were recorded on freely moving Göttingen minipigs instrumented with ITS telemetry implants (Konigsberg Instruments, USA), except for one signal, which was recorded on a dog instrumented with a DSI telemetry implant (Data Sciences International, USA). Recording duration was typically 27 hours long per file. All ECGs were acquired using lead II electrode placement.

### 1.2. Manual ECG analysis

In ECG signals from the database, 68 zones were selected with

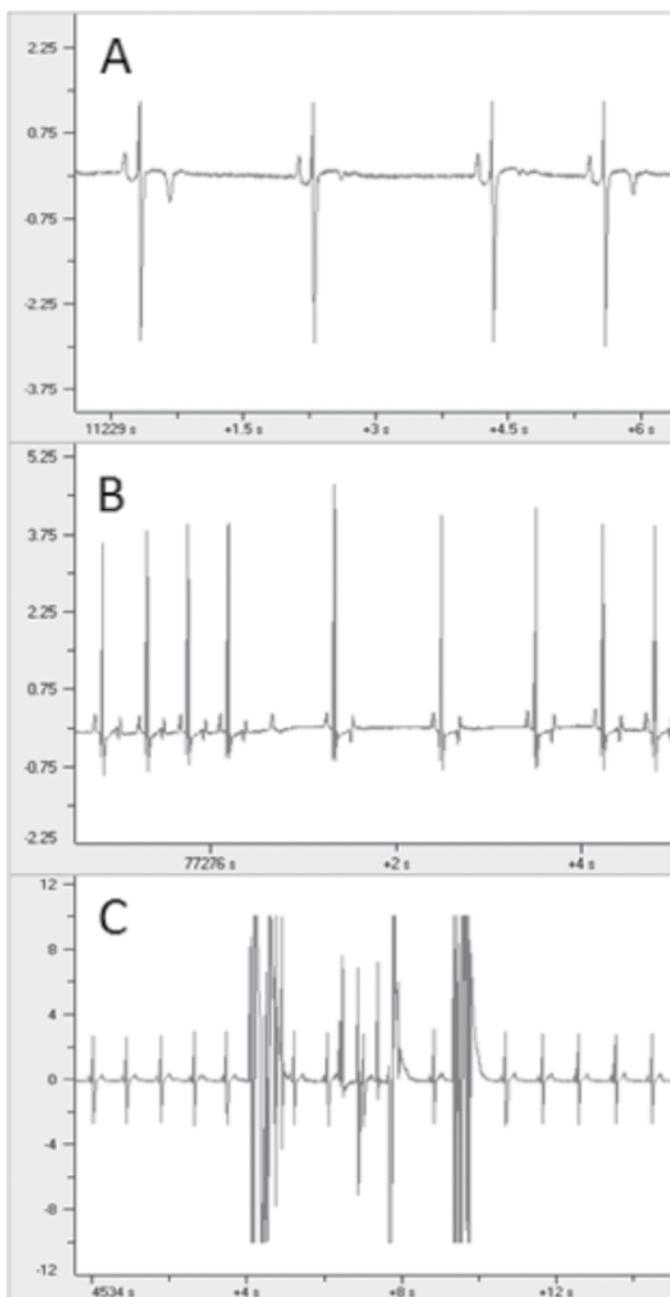


Figure 2. Examples of particular cases included in the validation data base. A: strongly changing T-wave shape; B: high variability of the heart rate; C: noise.

durations ranging from 60 to 360 seconds. Some selected zones contained low noise level and were chosen to cover the whole range of observed ECG morphologies. Other selected zones contained noise or particular cases, and were chosen to characterise the robustness and limits of the algorithm. Three representative examples are shown in Figure 2.

In each zone, all individual ECGs were manually annotated with P,Q,R,S,T points. Tolerance margins were set for each zone as the maximum error that is considered acceptable.

### 1.3. Principle of the ECG30s algorithm

Automated PQRST point detection was performed using ECG30s in NOTOCORD-hem™ version 4.2.0.261 (NOTOCORD Systems). The ECG30s analyser was designed to provide automated analysis during signal acquisition. The algorithm operated in two steps. The first step consisted of identifying QRS complexes in the ECG signal. Discrimination of true QRS complexes from false detections was mainly achieved following morphological criteria. The second step of the algorithm consisted in detecting P and T waves around the QRS complexes, and placing the PQRST points according to geometrical criteria. Since T waves in the minipig ECG are highly variable, the most challenging issue lied in positioning T end points. To deal with this issue, several T-end candidates were considered, as shown in Figure 3, and tracked over time. The algorithm then selected the candidate which best ensured QT duration stability, according to a statistical approach.

ECG30a also detected “abnormal beats”, defined by an abnormal variation of RR interval duration, i.e. RR shortening or prolongation. An abnormal beat was identified when the absolute value of:

$(RR \text{ Duration} / \text{average of the last 20 interval durations between 2 consecutive valid R-Peaks}) - 1$  exceeded a value specified in the module’s parameters as the “accepted RR duration change”.

Abnormal beats were not marked with PQRST points. This was a safeguard against misplacement of the T-wave end due to strong beat-to-beat heart rate-related QT variability.

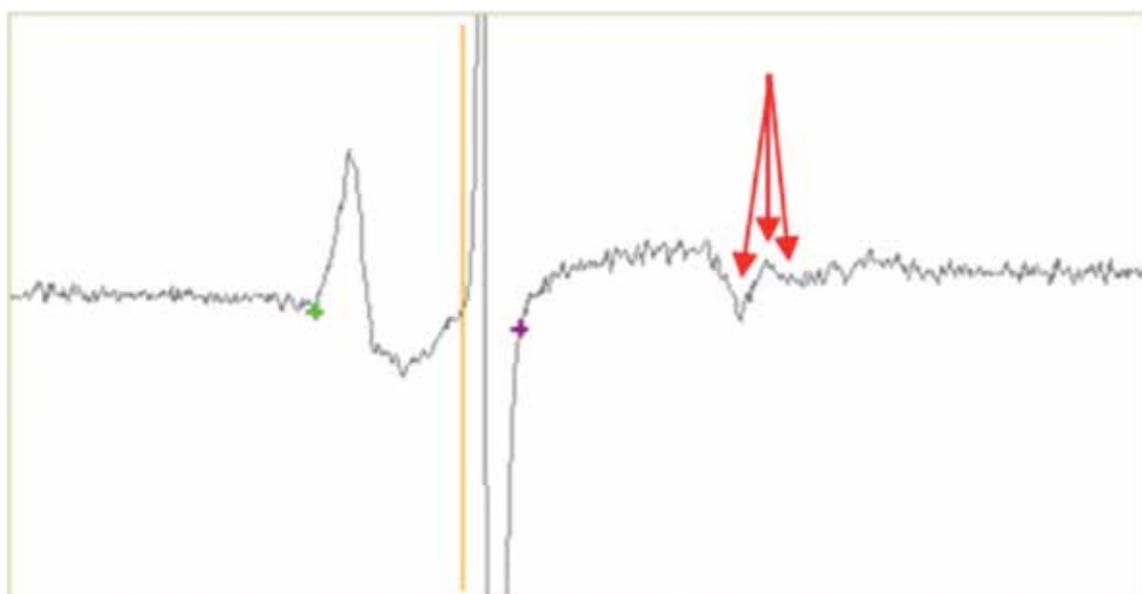


Figure 3. Example of possible T end candidates on a complex beat morphology (arrows).

## 1.4. Computation of performance criteria

The parameters used to assess the performance of the analyser are detailed in Table 1.

Table 1: Definition of performance criteria.

Name	Description	Formula
TP	Number of true positives in a zone. A true positive occurs when the automated analyser detects a point within the tolerance margins of the corresponding type of manually annotated point.	
FP	Number of false positives in a zone. A false positive occurs when the automated analyser detects a point, but it is not located within the tolerance margins of the corresponding type of manually annotated point.	
FN	Number of false negatives in a zone. A false negative occurs when the automated analyser fails to detect a point that was manually annotated.	
Se	Detection sensitivity measures ECG30s ability to detect each type of fiducial point.	$Se = \frac{TP}{TP + FN}$
P+	Positive predictivity of detection measures ECG30s ability to detect only fiducial points.	$P+ = \frac{TP}{TP + FP}$
$\mu e$	Average error between manual and automated analysis. Can be used as an index of accuracy of the analyser: - Positive when calculated points are primarily placed after reference points. - Negative when automated points are primarily placed prior to reference points.	$\mu e = \frac{1}{TP} \sum_{i=1}^{TP} e[i]$ where e is the difference between the reference and the automated points
$\sigma e$	Standard deviation of the error between manual and automated analysis is an index of ECG30s stability. A high $\sigma e$ does not necessarily reveal a bad automated positioning but a different one from the manual marking. Standard deviation $\sigma e$ must be considered to conclude on the relevance of the automated positioning.	$\sigma e = \sqrt{\frac{\sum_{i=1}^{TP} (e[i] - \mu e)^2}{TP}}$

## 2. Results

The overall result on the accuracy of fiducial point positioning is given in Table 2. Values are expressed as mean and standard deviation (in brackets) of the analysed files. In ECG zones with low noise level, manually positioned marks are found correctly by the software. The QT duration obtained with ECG30s is stable and not affected by the variability of the T-wave shape. In addition, the algorithm discriminates sharp P waves from the QRS complex (see example in Figure 4).

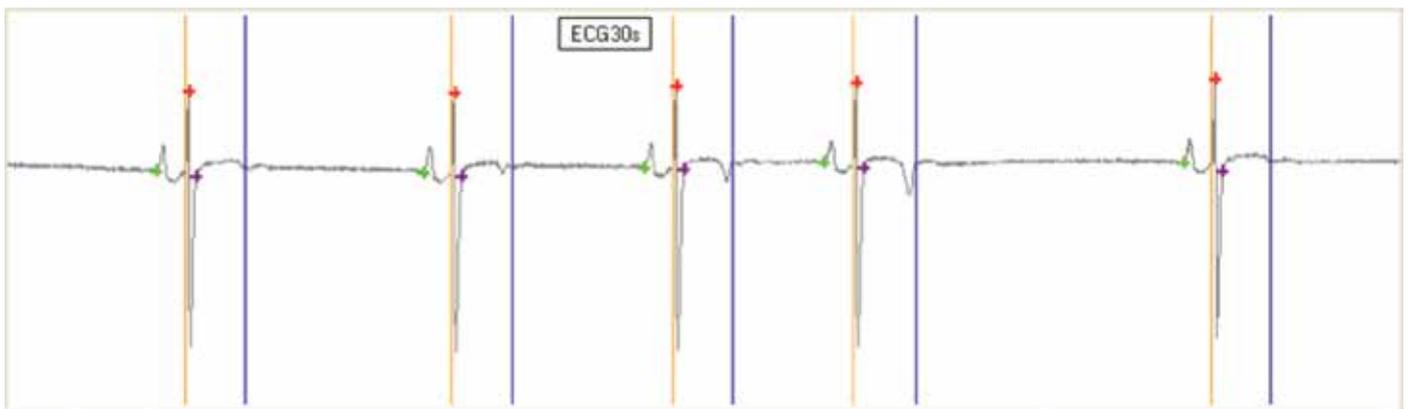


Figure 4. Example of analysis on changing T-wave morphologies in a minipig ECG signal. Detected p, Q, R, S and P points are indicated with a green cross, orange line, red cross, purple cross and blue line respectively.

Table 2: Detection performance for each fiducial point.

Fiducial points	Sensitivity (%)	Predictivity (%)	Average Error $\mu e$ (ms)	SD of Error $\sigma e$ (ms)
P Start	80.3 (21.3)	78.7 (18,7)	-2.72	2.44
QRS Start	85.5 (20.6)	83.8 (19.4)	1.15	2.19
R Peak	97.3 (8.1)	95.3 (3.4)	-0.04	0.80
QRS End	89.1 (17.2)	84.0 (16.1)	-3.80	1.41
T End	80.7 (21.3)	81.4 (18.3)	6.94	3.60

The obtained performance indicators are, however, lowered by the following particular cases:

- High variability of the heart rate

When abnormal beats are detected, marks are not placed (Figure 5), which decreases the detection sensitivity value. Abnormal beats sometimes result from arrhythmia. In this case, they can be analysed with ARR30a, another module of the NOTOCORD-hem™ software platform which is dedicated to arrhythmia detection (Koeppel, 2012).

- Zones with artifacts

Fiducial-point detection may be temporarily interrupted within a zone of artifacts. Furthermore, after the disturbed zone, a time period of few seconds is used to stabilise the algorithm during which no points are detected, thus decreasing the detection sensitivity value. However this process makes it possible to reduce erroneous detections and therefore increases the overall data quality.

- Presence of ECG morphology abnormalities

Particularities in ECG morphology are responsible for acceptable differences between manual and automated mark positioning in certain zones, leading to high-amplitude error average  $\mu$  and low-error deviation  $\sigma$  (each marking remaining consistent). An example is given in Figure 6 with a signal presenting T-wave offset notches at the end of the T wave.

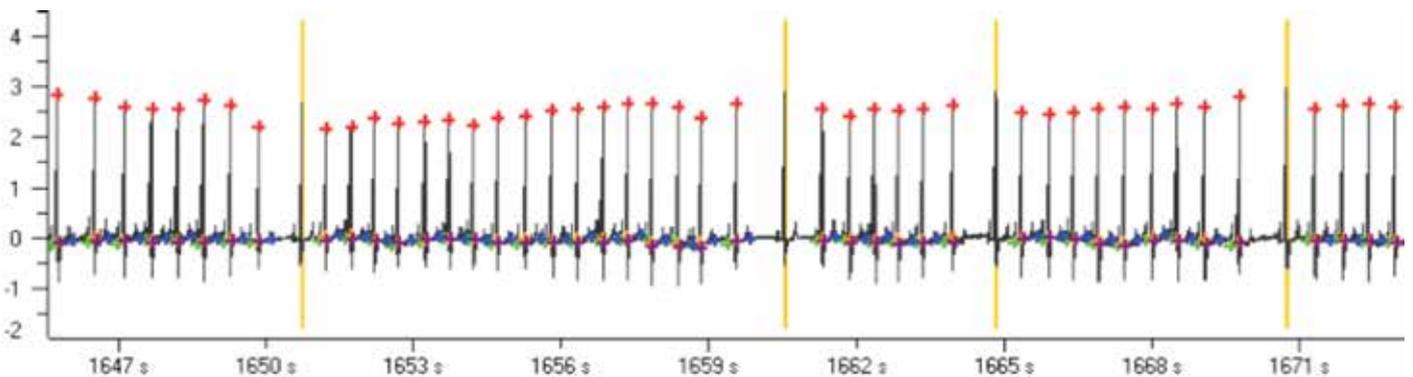


Figure 5: RR variability, leading to Abnormal beat identification (orange vertical lines) and missing PQRST marks.

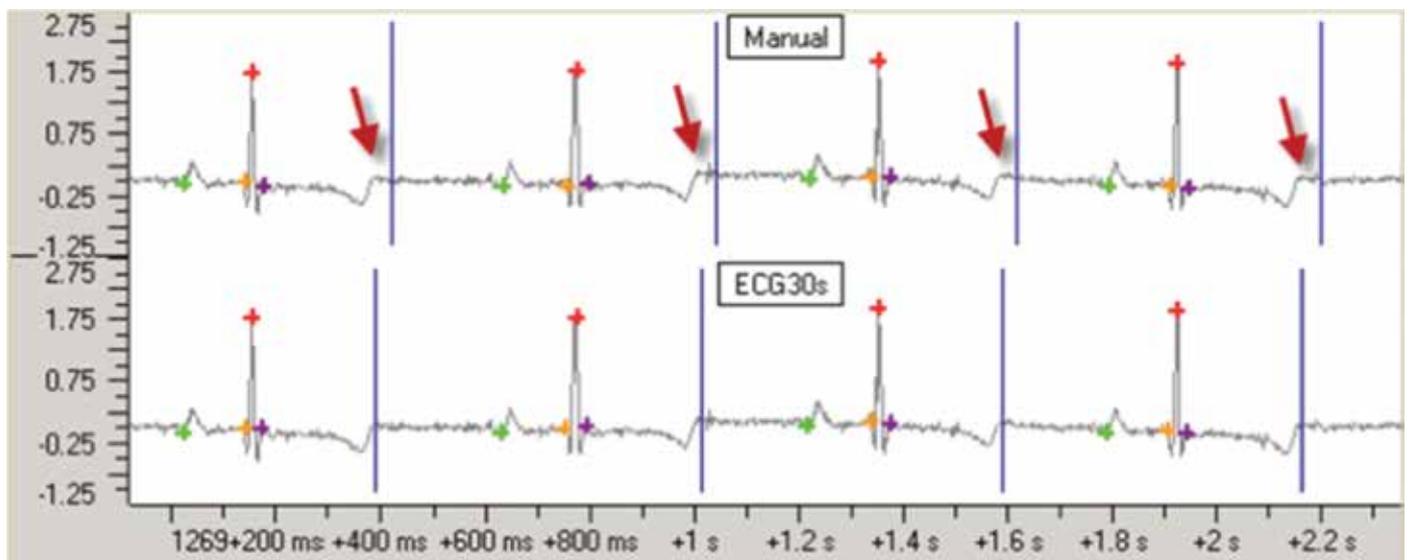


Figure 6: T-wave offset notches (arrows), leading to differences between manual (top) and ECG30s automated (bottom) marking and therefore a high error average. Since each marking remains consistent, the error deviation is low.

In addition, the ability of the analyser to measure changes in QT was evaluated. ECG was recorded in minipigs treated with various concentrations of dofetilide, a known hERG channel blocker. The T end was detected so that the prolongation of the QT interval could be clearly seen and accurately quantified. ECG30s analysis showed that QT interval increased with dofetilide concentration (Figure 7).

ECG30s is designed to analyse the ECG signal either during or after acquisition. Processing times were measured on two different files belonging to the validation database. Both files are 24 hours long but exhibit significantly different heart rates. Table 3 collects the obtained results.

Table 3: Processing time (minute'second") for the automated analysis of 24-hr-long minipig ECG recordings.

	Averaged heart rate	Processing time for 24 hrs
File 1	74.5 beat/s	5'50"
File 2	109.5 beat/s	9'30"

### 3. Discussion

The morphology of the P wave was typical for each individual animal and never changed obviously during the course of the experiment. However, the T-wave polarity shifted during the

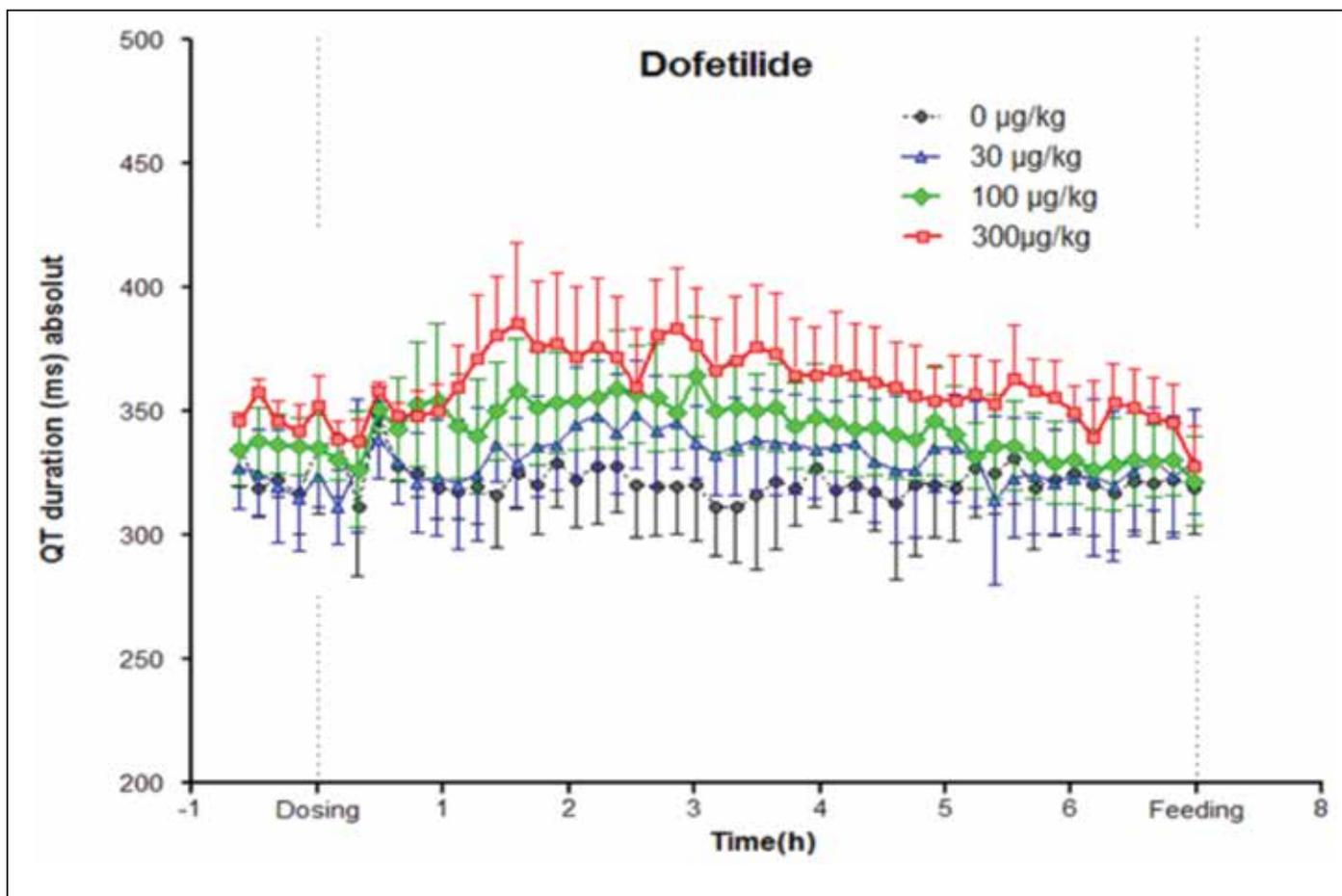


Figure 7. ECG30s analysis of the QT interval, showing dofetilide-induced QT prolongation. Each point represents a 10-minute average.

monitoring period without obvious reason. The latter was also apparent in other experiments in Göttingen minipigs (Eckenfels, 1988, Nahas, 2002); the reasons for these spontaneous changes are still unclear.

Of all T waves, 45% were positive, 42% negative and 13% bipolar. We could not see a relation between body position and T-wave polarity. Nevertheless, the T wave as recorded from lead II was always positive during periods of excitement in the animals.

With both positive and negative morphologies, the T end was easy to detect. With bipolar T waves in contrast, the end of T wave was often difficult to define and has caused problems to many algorithms used for ECG evaluation. However, ECG30s algorithm coped successfully with this issue, exhibiting a ~80% correctly-detected T-end score, thereby providing a time-saving and accurate tool for preclinical ECG analysis in the minipig.

#### 4. Conclusion

The automated real-time analysis of ECG signals with ECG30a and its specific QT stabilisation properties was found to ensure consistent detection of T end even with changing T-wave morphology in the minipig. It also discriminated sharp P wave from QRS complex, and processing was fast. ECG30s provides a solid basis for assessing drug-induced side effects in cardiac pharmacology, safety and toxicology studies in freely-moving and restrained minipigs.

#### References

- Eckenfels A, Schuler S. (1988). The normal electrocardiogram of miniature swine. *Arzneimittelforschung*, 38(2), 253-9.
- Koeppel F, Labarre D, Zitoun P. (2012). Quickly finding a needle in a haystack: A new automated cardiac arrhythmia detection software for preclinical studies. *J Pharmacol Toxicol Methods*, 66(2):92-7.
- Kuwahara M, Tsujino Y, Tsubone H. (2004). Effects of pair housing on diurnal rhythms of heart rate and heart rate variability in miniature swine. *Animals*, 53, 303-309.
- Laursen M, Olesen SP, Grunnet M, Mow T, Jespersen T. (2009). Characterization of cardiac repolarization in the Göttingen minipig. *J Pharmacol Toxicol Methods*, 63(2):186-95.
- Markert M, Stubhan M, Mayer K, Trautmann T, Klumpp A, Schuler-metz A, Schumacher K, et al. (2009). Validation of the normal, freely moving Göttingen minipig for pharmacological safety testing. *J Pharmacol Toxicol Methods*, 60(1), 79-87.
- McAnulty PA. (1999). The value of the minipig in toxicity and other studies supporting the development of new pharmaceuticals. *European Pharmaceutical Contractor*, 82-86.
- Stubhan M, Markert M, Mayer K, Trautmann T, Klumpp A, Henke J, Guth B. (2008). Evaluation of cardiovascular and ECG parameters in the normal, freely moving Göttingen Minipig. *J Pharmacol Toxicol Methods*, 57(3), 202-11.
- van der Laan JW, Brightwell J, McAnulty P, Ratky J, Stark C. (2010). Regulatory acceptability of the minipig in the development of pharmaceuticals, chemicals and other products. *J Pharmacol Toxicol Methods*, 62(3), 184-95.

# Minipig Activity – group housing vs single housing

Adrian Zeltner and Helle Lorentsen

*Ellegaard Göttingen Minipigs*

Does housing minipigs in groups have an impact on activity compared to housing them singly?

Do group-housed minipigs benefit more or less from enrichment than singly-housed minipigs?

To find out, we put up a camera in front of two pens and filmed day and night for 3 days.

Pen A: 1 female minipig.

Pen B: 5 female minipigs.

All aged 3 months.

A metal chain and a rubber bite stick were available in both pens.

The minipigs in both pens were able to have snout contact with their neighbours.

The light was on from 6 a.m. to 6 p.m.

Each day the enrichment was changed as follows:

Day 1: straw

Day 2: straw and bucket in chain to play with

Day 3: straw and a pyramid to mount and push around

## The A-minipig

Eats more slowly than B-minipigs.

Is awake up to 1 hour before B.

Falls asleep later than B.

Not very interested in B-minipigs.

Afraid of bucket and pyramid the first hours.

Plays with the bucket for hours in the afternoon.

Pays less attention to the bucket and pyramid than B.

Not very interested in straw, because of leftovers from the day before.

## The B-minipigs

Sleep in a pile – wake up when light is turned on at 6 a.m.

Enjoy provision of bedding because of no leftovers from the day before.

Play almost immediately with the bucket in chain. Play a lot with it all day long.

Provision of straw makes the bucket uninteresting – after a while straw sticks to the bucket which enhances the “fun-factor” of the bucket considerably.

Not afraid of pyramid – they mount it and push it around a lot.

Provision of straw makes the pyramid uninteresting.

## Conclusion

The Göttingen Minipig is a social animal which should be housed in groups. In Ellegaard’s barrier facilities the minipigs are always group housed – except for sows during farrowing and lactation and boars used for breeding, both of which have to be housed separately.

This limited (non-scientific) project shows us that minipigs in groups benefit more from enrichment than singly-housed minipigs.

If for some documented reason you have to keep minipigs housed separately, make sure they have enrichment and contact with staff. You can also let them take short walks in the corridor.

## General observations

Minipig A is awake and up 1 hour before B.

The A-minipig goes to sleep up to 1 hour later than B-minipigs.

Staff entering the room distracts their activities.

B-minipigs are more active and interested in surroundings, enrichment and other minipigs than the A-minipig.

The A-minipig uses the metal chain a lot, and often lies in the corner.

Straw is superior to all other types of enrichment.

The A-minipig is afraid of the bucket and pyramid for several hours.



B: seeing other minipigs playing with the bucket seems inspiring



A resting, not interested in straw – B active and curious



B: Pigs in a pile

Male minipigs can be group-housed up to the age of 1½ year – but only if they are used to each other. When ordering minipigs don’t forget to mention how they will be housed in your facility. By doing this you can avoid having to make new groups upon arrival.

# SOT Exhibitor Sponsored Sessions

Sessions at the annual SOT Meeting in San Antonio, TX, March 10-14 2013 presented by Marshall BioResources and Ellegaard Göttingen Minipigs:

## Juvenile Safety Assessment in Göttingen Minipigs

Monday, 11 March 2013 , 3:30 PM to 4:30 PM, Room 007 B

This session consists of two talks:

### **Immunodevelopment of the Göttingen Minipig**

Speaker: Geertje van Mierlo, TNO Triskelion, Zeist, The Netherlands

### **The Minipig in Juvenile Toxicity Testing**

Speaker: Donald Stump, Wil Research, Ashland, Ohio

This session describes the benefits of the Göttingen Minipig as an additional model in juvenile safety assessment studies; the physical and sensory development of piglets; and the potential for minipigs in evaluating the toxicity of certain compounds on the development of the nervous system, reproductive system and immune system.

## The Value of the Göttingen Minipig in the Development of New Biotherapeutics

Tuesday, March 12, 2:45 PM to 3:45 PM | Room 007 A

This session consists of two talks:

### **Filling the Gaps: the Göttingen Minipig in safety testing of biopharmaceuticals – genome sequencing, mRNA and protein expression**

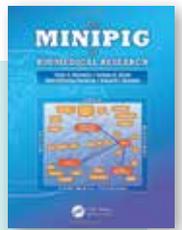
Speaker: Ulrich Certa, F. Hoffman La-Roche, Basel, Switzerland

### **The Göttingen Minipig as model for the pharmacokinetic assessment of monoclonal antibodies**

Speaker: Wolfgang Richter, F. Hoffman La-Roche, Basel, Switzerland

These presentations discuss the Göttingen Minipig as a potential model in preclinical testing of biopharmaceuticals. Recent studies have demonstrated some similarity to human sensitivity to various biologically-derived compounds, including certain monoclonal antibodies. Sequencing of the Göttingen Minipig genome means genomic level target validation, and potential cross-reactivity, is now possible.

## Reviews of the book “The Minipig in Biomedical Research”



The book “The Minipig in Biomedical Research” was published in December 2011 and is an excellent manual for matters pertaining to minipigs.

Whether you have much experience with minipigs or not you will benefit from reading this book or parts of it.

The book includes 41 chapters organised into 8 sections:

- Origin and Management of the Minipig
- Welfare and Experimental Usage of the Minipig
- Pharmacology and ADME Studies in the Minipig
- Safety Assessment in the Minipig – Principal Body Systems
- Genetics and Immunology
- Disease Models
- Regulatory Perspective and Use of the Minipig in Developing Marketed Products
- New Horizons and perspectives

*“Overall, this book will be a very useful reference source on the minipig for anyone involved with this species in biomedical research.”*

Book review by Guy Healing

*“There is a great need for reference texts concerning the use of minipigs in biomedical research and this textbook clearly aims to help fill the void.”*

Book review by Kristy Conn

You can find the book reviews on our website [www.minipigs.dk](http://www.minipigs.dk)

## Meeting calendar

Name	Date	Location
SOT ToxExpo – Society of Toxicology	10-14 March	San Antonio, Texas, US
IAT - Institute of Animal Technology	12-15 March	South England
BTS – British Toxicology Society	9 April	West Midlands, UK
Scand-LAS	17-20 April	Tallinn, Estonia
6th Juvenile Toxicity Symposium	16-17 May	Beerse, Belgium
FELASA/SECAL	10-13 June	Barcelona, Spain
SPS – Safety Pharmacology Society	16-19 September	Rotterdam, The Netherlands

### Europe and Asia

Ellegaard Göttingen Minipigs A/S  
Sorø Landevej 302, DK-4261 Dalmose  
Denmark  
Tel. +45 5818 5818  
[ellegaard@minipigs.dk](mailto:ellegaard@minipigs.dk)

### North America

Marshall BioResources  
North Rose, NY 14516, USA  
Tel. +1 315 587 2295  
Fax +1 315 587 2109  
[infous@marshallbio.com](mailto:infous@marshallbio.com)

### Japan

Oriental Yeast Co. Ltd.  
3-6-10, Azusawa, Itabashi-ku  
Tokyo, 174-8505, Japan  
Tel: +81 3 3968 1192  
Fax: +81 3 3968 4863  
[fbi@oyc.co.jp](mailto:fbi@oyc.co.jp)

[www.minipigs.dk](http://www.minipigs.dk)