Automated blood sampling in minipigs

By Malene Schrøder[1] and Sten Velschow[1]

[1]Fluisense ApS, Copenhagen, Denmark

It is often a requirement during animal trials to do repeated blood sampling to measure changes either in the concentration of biomarkers or of a dosed compound in the blood. A general goal of animal studies is to minimize the number of animals used while providing high quality data. A general limitation in animal studies is the number of blood samples that can be collected from one given animal contra the number of timepoints the scientist would like.

Introduction

Repeated blood sampling is required in many research settings, e.g. during PK/PD studies and safety pharmacology studies. For some specific study designs it is necessary to have satellite groups of animals for blood sampling as this procedure affects negatively other sensitive end-points, like telemetry - or behavioral data.

Currently, manual procedures for serial sampling in larger animals (dogs, pigs and NHP) requires lifting the animal into a sling, training the animal to sit on a table, other restraint procedures or eventually implanting long-term intravenous catheters.

Serial manual blood sampling includes the following common challenges:

- It is labor-intensive, time consuming and may introduce human errors. It requires involvement of numerous staff to both fixate the animal and manually handle each blood sample.
- Complex logistics are involved when handling wet blood samples in terms of labelling, centrifugation, and freezer facilities.
- Data quality is affected by many possible sources of error during collection, handling, and storage of samples.
- Reproducibility of data is challenging, as the exact test set-up is dependent on diligent time schedules and success during each sampling occasion.
- Occupational health is a factor when handling large animals, working round the clock and being under time pressure.
- Animal welfare and ethics dictate how many samples you can collect from one animal in a trial.

Automated serial blood sampling devices provide an alternative to manual serial blood sampling. There are different products on the market which are specifically aimed for smaller laboratory animals like rodents. Culex® (Bioanalytical Systems, Inc.) and ABS2 (Instech Laboratories, Inc.) are examples of automated samplers which share the common feature that they withdraw blood from a tethered, freely-moving animal. These systems are not directly usable for larger animals.

For larger laboratory animals, i.e., rabbits, NHPs, dogs and minipigs, Fluispotter®, a wearable automated blood sampling device which allows for up to 20 hours of unattended serial sampling of maximum 20 blood samples, can be used.

Implementation and use

Equipment

Fluispotter is a small device which requires minimal investment in infrastructure in your animal facility. In Figure 1. you see Fluispotter, which consists of:

A Control System, which is a reusable, rechargeable, programmable wearable equipment
B Software for programming of Control System and generation of data log for GLP records
C Cartridge, which is a sterile consumable for single use. The Cartridge has a 6 ml reservoir for flushing solution, and it collects blood samples as dried blood spots
D Catheter, which is a sterile consumable for single use; 45 cm long, multi-lumen for recirculation and limited blood loss

When operational, Fluispotter weighs 75 grams and is mounted on the back of the animal.

Figure 1
From left: Fluispotter Catheter with markings, attached to Fluispotter Cartridge. Fluispotter Control Unit and to far the right the assembled Fluispotter. In front, paper-reel with dried blood spot samples.

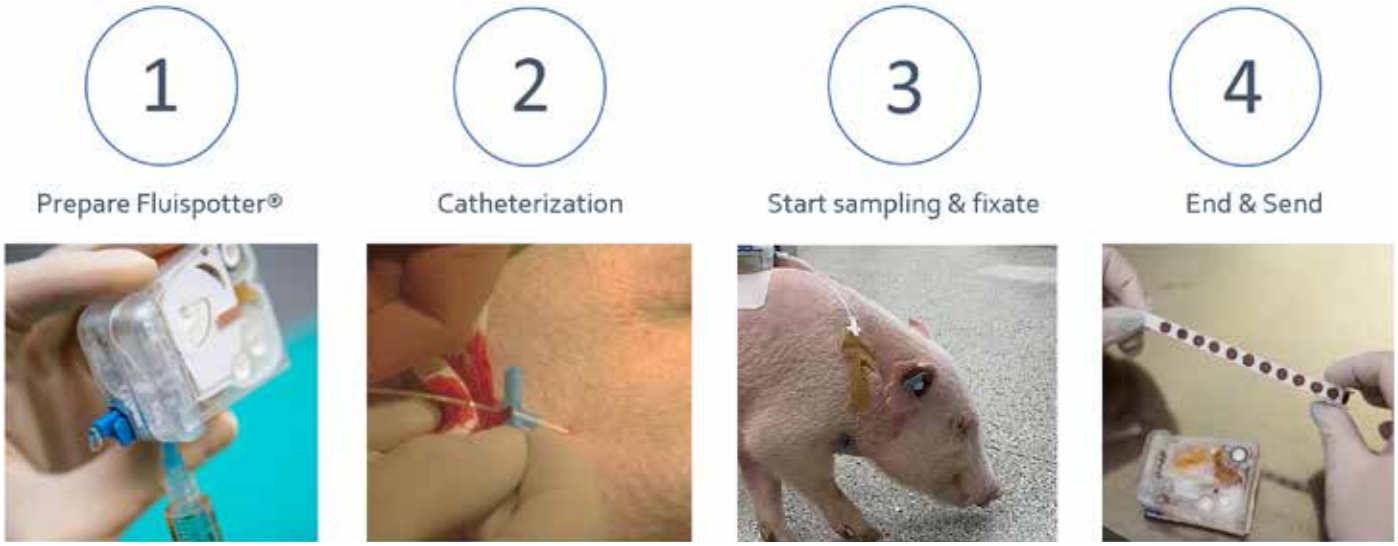


Figure 2
Fluispotter procedure in 4 easy steps.

Methods
The whole sampling procedure is completed in four overall steps (Figure 2).

1. Preparation of Fluispotter

The sampling schedule is created in Fluiconnect™ software, which must be installed on a PC. Via Fluiconnect the Fluispotter Control System is programmed and charged.

Fluispotter Cartridge™ and Fluispotter Catheter™ must be preloaded with flushing solution before use; 4% Sodium Citrate solution is recommended.

Fluispotter Control is clicked together with Fluispotter Cartridge and the system is ready for use.

2. Catheterization

Catheter placement is performed using sterile principles while the minipig is placed under full anaesthesia in a supine position for a short surgical procedure.

Fluispotter Catheter must be placed in a large enough vein, preferably the jugular vein. A guide catheter is introduced into the jugular vein via the Seldinger technique:

- a guidewire is introduced into the jugular vein via an 18 Ga needle
- a small skin incision close to the guide wire is made using a scalpel
- a guide catheter is placed over the wire
- the guide wire is removed, and the guide catheter flushed with sterile salt water
- Fluispotter Catheter is placed through the guide catheter into its desired depth, which is dependent on the size of the minipig
- the catheter is connected to the Fluispotter Cartridge
- the guide catheter is then carefully removed from the vein while ensuring the Fluispotter Catheter stays in place

An alternative is to place a 16 Ga Peel-Away Introducer directly in the jugular vein, placing the Fluispotter Catheter and removing the peel-away.

To ensure that the Fluispotter Catheter is not misplaced during the sampling period, it should be fixed to the skin. This can be done either by suturing a catheter clamp to the skin or alternatively with a strong plaster like Fixumull®.

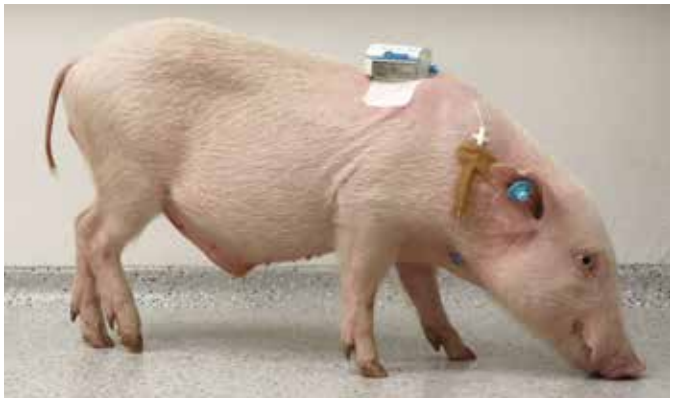
3. Start sampling and fixate Fluispotter

Fluispotter is started by pushing the button. Once the catheter is fixed to the skin and Fluispotter is mounted on the back (see Picture A) it is recommended to bandage the neck and chest using a flexible self-adherent bandage.

4. End & Send

Once Fluispotter has completed the desired sampling profile, the animal must be restrained shortly (see Picture B) to remove the catheter from the vein and detach the Fluispotter and the adhesive patch from the back of the pig.

The cartridge contains a paper strip with the dried blood samples. The paper strip is easily removed from the cartridge



Picture A
Göttingen Minipig wearing Fluispotter.

and can be stored in a dry place at room temperature until analysis is performed in the lab. The cartridge and the catheter should be disposed as medical waste.

The Control Unit requires no maintenance, but regular recharging of the battery.

Output

Data log

When reconnecting Fluispotter Control Unit to the PC, it will be possible to obtain a data log showing exactly when the blood samples were collected and the exact volume of each sample.

Dried blood spots

Fluispotter collects up to 20 volumetric dried blood spots (DBS) of 3-10 µL over the course of up to 20 hours. The accuracy of the blood sampling volume is ± 0.3 µL (1).

Due to the design of the catheter tip and the constant recirculation of blood, carry-over is < 5% when sampling at 10 min. intervals. Method validation experiments must be performed to establish the validity of the analytical assay for the compound you want to measure in the blood sample.

Discussion

Dried blood spots

DBS was introduced in the early 1960's in neo-natal screening for metabolic disorders. The main feature for the use of DBS was the low blood volume requirement, but the technique has proven valuable as a very easy way of blood sampling and a very robust way of storing blood samples.

Since the early 2000's the development of LC-MS/MS equipment and immunoassays provided very sensitive assays, that has initiated an intense development of DBS based assays as the ability to generate analytical results from small blood samples could improve the ethical use of laboratory animals, as it allowed less discomfort and limited the number of animals used.

The use of DBS did experience a set-back in drug development as FDA, in the draft Guidance for Industry Bioanalytical Method Validation of 2013, stated that exposure data based on DBS should be supported with correlative studies based on traditional sampling (2). The statement was repeated in the final version of 2018 (3). However, because of the advantages of DBS compared to wet samples, the use and implementation is still ongoing, mainly in research and clinical applications and in the area for home testing purposes. Today, numerous biomarkers, small molecules, large molecules, and antibodies can be recovered from DBS.

The ICH Guidance E14/S7B for the non-clinical evaluation of QT/QTc Interval and Prolongation and Proarrhythmic Potential (4)

The recently published final ICH Guidance document considers it best practice to preferable use the same animal species to obtain telemetry data and complementary information on systemic exposure levels (toxicokinetic) for in vivo QT studies. The Guidance document used the principles of 3R as an argument for this recommendation.

Due to the limited availability of dogs and NHP and the general understanding that minipigs are a good model for humans regarding heart monitoring, minipigs are being more widely used in telemetry studies.

As Fluispotter is wearable and automated animal trials can be performed without having to worry about human presence stressors or constraint of the animals during sampling thereby affecting the outcome of the trial. A study in Beagle dogs (5) shows cortisol levels significantly lower than the normal reference interval, indicating that Fluispotter® opens new ways of reducing pain, fear, and discomfort for animals in animal trials, and gives the research community a new powerful tool to further 3R efforts.

Fluispotter is the best available solution for larger laboratory animals to refinement and reduction, better animal welfare and improved quality of data.

Picture B
Gentle handling of Göttingen Minipig during removal of Fluispotter.

REFERENCES

1 Adhikari et. al. (2020): Fluispotter, a novel automated and wearable device for accurate volume serial dried blood spot sampling. Bioanalysis. DOI: https://doi.org/10.4155/bio-2020-0048.
2 Guidance for Industry Bioanalytical Method Validation DRAFT GUIDANCE (2013).
3 Guidance for Industry Bioanalytical Method Validation Final (May 2018).
4 E14 and S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential - Questions and Answers. ICH Guidance (Aug 2022).
5. Ollerenshaw et al. (2022): A novel device for serial venous blood sampling in a canine model. Journal of pharmacological and toxicological methods. DOI: https://doi.org/10.1016/j.vascn.2022.107155.