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NC3Rs

National Centre for the Replacement, Refinement and Reduction of Animals in Research

Automated blood sampling and the 3Rs

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Abstract

Automated blood sampling (ABS) systems have been increasingly employed in biomedical research since the mid-1990s for use in pharmacokinetic, safety pharmacology, metabolism, biomarker and other studies in industry and academia. Originally conceived with the purpose of reducing animal use and stress, this article reviews the contribution of ABS systems to the 3Rs concept of Russell and Burch (1).

Keywords: automated blood sampling, catheter, reduction, refinement, stress, vascular puncture.

Background

Historically, when blood samples from animals have been required for various scientific purposes, the samples have been withdrawn directly from the required blood vessel or tissue with a needle and syringe, or through other means, such as decapitation (see www.nc3rs.org.uk/bloodsamplingmicrosite). Anaesthesia is used to minimise any pain and/or distress, unless to do so would cause more discomfort for the animal than the method of blood collection itself. Although these blood collection techniques are still in use today, an increasing number of laboratories are using indwelling, surgically implanted catheters or vascular access ports (see Glossary) for blood sampling, particularly when multiple samples are required from the same animal (2-5).

In the early 1990s, DiLab was approached by Novo Nordisk to devise a way to automate the blood sampling process in rats as a means of reducing both animal use and the stress caused to the animals from repeated handling, restraint and vascular puncture. Several years later, the first commercial device, the AccuSampler®, was introduced (Figure 1). In the following years, devices from other manufacturers were brought to the market.

Figure 1. *A mouse connected to the AccuSampler® via a catheter. A spring tether is used to protect the catheter and extension line leading up to a swivel, which is mounted on a spring-balanced lever arm.*

To date, ABS systems have been employed for sampling in numerous species including mice, rats, hamsters, guinea pigs, beagle dogs, mini-pigs and macaques.

The use of ABS systems has been shown to (6-9):

- result in lower levels of various stress markers in blood samples, such as corticosteroids and glucose
- allow for a reduction in animal use
- provide higher quality data, and
- save time and staff resource in the laboratory, allowing scientists to investigate alternate laboratory methods and procedures and improve throughput.

The ABS process

A thorough understanding of the physics of fluid movement through tubes and blood vessels of various diameters, and the biological limitations to maintaining normal physiology in blood-sampled animals (10), allows the blood sampling process to be automated. Regardless of the animal species being sampled, the process begins with having ready access to the required blood vessel by means of a surgically implanted catheter or vascular access port. Typically, catheters are used for small animals (e.g. rodents) and studies of 24-48 hour duration. In rats, the site of implantation is usually the jugular vein or carotid artery, but other vessels (e.g. portal vein, femoral artery) are also used. Vascular access ports are generally used for larger animals (e.g. dogs, pigs and macaques) or when a protocol requires longer-term use of an animal.

The catheter implantation procedures take place under general anaesthesia and aseptic or sterile conditions. Following surgery, the animals are administered analgesics to minimise pain and are allowed a period of time to recover before studies are commenced (8,11,12). During this time, the catheters are kept open and free of blockage (patent) by periodic flushing with heparinised saline or by filling the catheter with a slightly viscous solution containing an anti-coagulant (e.g. heparinised glycerol).

With increasing frequency, animals are implanted with multiple catheters to allow for the administration of test compounds as well as blood sampling (2,7,9,13,14) without the need for repeated handling, restraint and vascular punctures, which can result in increased stress and pain to the animal (see Refinement).

ABS systems are comprised of one or more pumps and valves to direct sampled blood to a refrigerated collection vial, as well as to keep the catheter flushed and return fluid to the animal (Figure 2). For most studies, the system is filled with sterile heparinised saline (approximately 20 IU/ml). Prior to the experiment, the animal is fitted with a tethering device, which allows it to move as freely as possible in its home enclosure whilst avoiding the withdrawal and injection lines becoming contaminated or damaged (Figure 1).

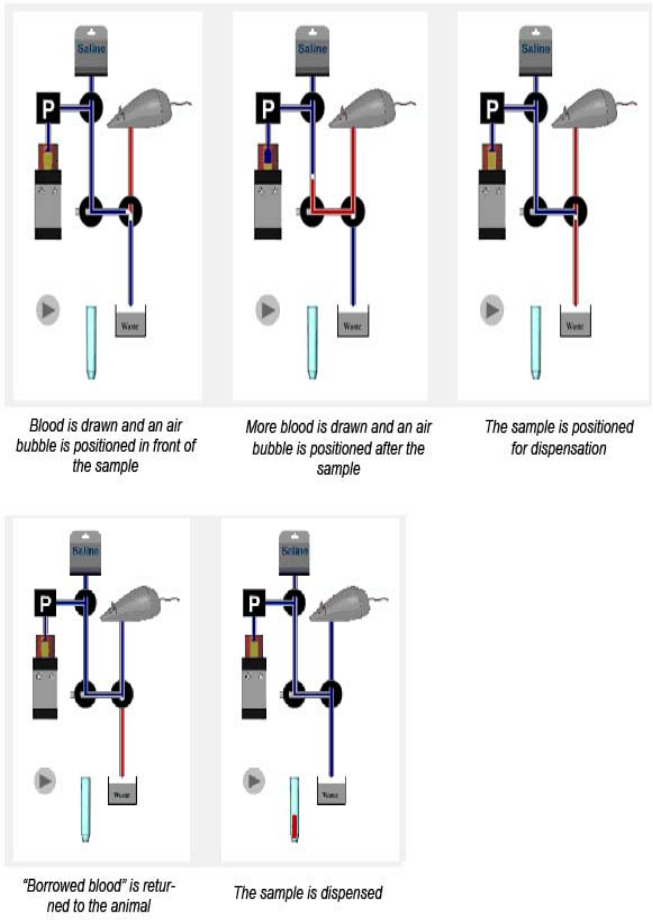

Figure 2. *Schematic illustration of the blood sampling procedure using the AccuSampler®. With this particular device, air bubbles are used to avoid mixing of blood and saline in the tubing system.*

The automated system means that defined blood volumes can be withdrawn at pre-programmed times. Initially, blood is drawn through the catheter and into the tubing system of the ABS device. As the tubing and catheter are filled with saline, the first volume of blood drawn is mixed with saline and therefore diluted. This initial volume is either flushed to waste, or returned back

to the animal. It should be noted, therefore, that when comparing data from catheterised animals connected to ABS systems with direct vascular puncture methods of blood sampling, blood concentrations from the catheterised animals may be 5-15% lower due to the initial mixing that takes place in the catheter. This is minimised by taking larger sample volumes, as well as by taking larger volumes of blood before the actual sample.

The second step involves removing the actual sample and placing it in a refrigerated vial. The sampling procedure is completed by flushing the system with saline to minimise potential carryover of compound from sample to sample. A volume of saline equivalent to the blood sample volume is returned to the animal so that proper fluid balance is maintained. In most cases, this fluid replacement also eliminates the need for additional animals to supply donor blood (see Reduction). In the ABS protocol, small amounts of heparinised saline (10-20 µl) are flushed to the animal at periodic intervals to maintain catheter patency.

ABS and the 3Rs

ABS systems can potentially contribute to two of the 3Rs, namely refinement and reduction.

Refinement

The scientific literature extensively documents the effects of handling, restraint and blood collection on laboratory animals, including increases in heart rate and blood pressure, elevated concentrations of hormones such as corticosterone, and alterations to immune system responses (15). The observed changes in physiological parameters suggest that these procedures are stressful for the animals and may introduce confounding variability to scientific data, potentially decreasing study reliability.

Using ABS equipment makes the blood sampling procedure less stressful for the animal and more accurate than manual blood sampling, by avoiding repeated handling and restraint and the potential for pain and/or discomfort from repeated anaesthesia and/or vascular puncture (6). This is particularly desirable where many samples are required from the same study animal. Although there have been very few studies investigating the refinement potential of ABS, Royo *et al.* found that the AccuSampler® was able to draw blood samples from male Sprague-Dawley rats without inducing corticosterone release (8).

There are animal welfare concerns surrounding the use of ABS, mainly because the equipment necessitates an invasive procedure to install the catheter and connect the animal to the device, as well as surgical anaesthesia, a well-known stress factor in rodents and other laboratory animals (3,14,16-21). In the Royo *et al.* study, a stress response (characterised by an increase of the total release of corticosterone and an increase of fecal immuno-reactive corticosterone metabolites) was observed after implanting jugular vein catheters in the rats under general isofluorane anaesthesia for connection to the AccuSampler®. However, the rats recovered quickly, indicating that results obtained using ABS in pharmacological and physiological studies may not be biased by a strong acute stress response in the animals following surgery.

Sampling via an indwelling catheter is generally considered to be less stressful and to cause fewer changes in blood variables than sampling with a needle and syringe, with or without anaesthesia (22-24). Recent blood sampling methods may compare more favourably: for example, Fluttert *et al.* (25) found comparable concentrations of corticosterone in blood collected from jugular vein catheters and blood from incision and gentle milking of the lateral tail vein (tail nick) in rats. However, Vahl *et al.* (26) found that adrenocorticotropic hormone (ACTH) concentrations were greater in blood collected via tail nicking compared with inferior vena cava catheters, possibly reflecting the longer time period required to obtain the sample from the tail vein. It is not known whether there is a difference in corticosterone or ACTH concentrations between catheter sampling alone and ABS.

Venous catheters have the advantage of simplifying repeated sampling, especially where large blood volumes are needed. While removing 3ml of blood or more from rats in a short period of time increases plasma corticosterone levels (3), the availability of a venous catheter allows for either the transfusion of donor blood (27) or the return of erythrocytes suspended in sterile saline, such that hypovolaemia and anaemia (see Glossary) can be prevented during serial sampling. The AccuSampler® compensates for the amount of blood sampled by replacing the same volume of saline solution to maintain fluid balance.

Indwelling venous catheters can be associated with complications, such as septic thromophlebitis and generalised sepsis (see Glossary) in rodents, which can be avoided to some extent by sterile surgical technique (23,28). There is a legitimate concern that the implantation and presence of the catheter is itself a source of chronic irritation or inflammation (2,11). Careful choice of catheter material, lock solution (see Glossary) and catheterisation site may help address these issues (12,29,30).

Chronically catheterised animals, particularly smaller species such as rats, are often restrained by some form of harness, swivel and tether along which the catheter runs; this is the case with the AccuSampler® (Figures 1 and 3). Harnessing has been shown to stress rats (31) and tethering restricts the normal movements of animals, such as rolling over and lying on their backs. Furthermore, catheterised and tethered animals are usually housed singly for the duration of the study to prevent possible damage of the equipment by cage mates (25,26), thus adding to the stress and severity of the procedure (23). Where single housing is necessary, this need not preclude providing environmental enrichment to meet the animal's behavioural needs, such as bedding material and chew blocks for rodents.

In conclusion, the refinement potential of ABS is not well studied and will most likely depend on the specific circumstances of the study (e.g. the number, volume and quality of blood samples required). A well-controlled comparison of the animal welfare implications of ABS versus common manual methods of blood collection would therefore be of considerable value. A number of indices of animal welfare would need to be recorded in order to make the comparison, including heart rate, blood pressure and temperature using telemetry (32), hormone levels in plasma, urine or faeces, body weight and behaviour.

Figure 3. *A harness used for rats. Similar harnesses are available for other species.*

Reduction

Depending on the animal model and protocol used, significant reductions in animal use (e.g. 50-80%) can be achieved by using ABS systems, which is both ethically and financially desirable. For example, some mouse studies require blood sampling at six to eight time points. If performed manually, this type of study would typically require 18-24 mice, compared with only three mice using ABS. In the manual study, traditionally only one sample is taken per mouse and time point, often through cardiac puncture. With ABS, all six to eight time point samples can be taken from the same mouse. This is possible since the instrument can handle very small sample volumes (e.g. 5-50 µl), which is not possible to do accurately when sampling manually.

Animal use can be further reduced by using the same animal for multiple studies, assuming that assay requirements are within the individual institution's

guidelines for the amount of blood removed at specific time points. Once the catheter is implanted it is very easy to do repeated sampling of small blood volumes using ABS. One example of this is "crossover" studies, where a test compound is administered intravenously to an animal and, after an appropriate washout and recovery period, the animal is used again for an oral dose of the same compound (9). Another possibility is using the same animal in a control study as well as in an actual test. For example, a glucose tolerance study may be performed on the same animal without a test compound and again, following recovery, after administering a test compound. Using ABS systems can also eliminate the need for donor animals which are used to supply blood to the test animal to maintain fluid balance.

The combination of reduced handling stress and the robotic involvement of the ABS device, taking precise volumes at reproducible flow rates, means that coefficients of variation are typically improved in pharmacokinetic and metabolism studies using ABS compared with manual sampling methods. Additionally, because the animals are not handled and are freely-moving (although tethered), physiological levels of stress markers such as corticosteroids, ACTH, and cortisol can be determined. The lower physiological basal rates of these stress hormones do not contribute to abnormal metabolism of test samples. Even simple glucose levels have been found to be significantly lower in ABS animals than animals sampled by vein puncture (unpublished data).

Reduction can also be achieved in safety pharmacology studies, where physiological parameters (such as blood pressure and heart rate) are monitored with telemetry and then correlated to drug levels. Historically, this would require two separate studies, as the animals' physiology would be greatly affected by the stress responses associated with handling and restraint for manual blood sampling. The use of ABS systems allows the two studies to be performed in the same animal, thereby reducing animal use and providing higher-quality data by minimising inter-animal variability, since all parameters are measured in the same animal.

An important consideration in pre-clinical drug development is the time and labour involved in the serial sampling of small blood volumes from small animals, such as rats, for the duration of pharmacokinetic studies. Using ABS can speed up this process, enabling increased throughput of screened substances. Although this could potentially lead to increased animal use overall, the outcome is usually faster screening of the substances awaiting evaluation and not screening of an increased number of substances.

Conclusion

ABS systems have a number of advantages when compared with manual blood sampling methods, including improved data quality and reduced animal use, the potential for reduced animal stress during sample collection, and time and staff resource savings. For each individual experiment, these advantages must be weighed against the need for the animals to undergo surgery with anaesthesia, tethering and single-housing.

Acknowledgements

We would like to thank Dr Mark Prescott at the NC3Rs for his input and assistance with the Refinement section.

Glossary

Anaemia: A reduction below normal in the number or volume of red blood cells (erythrocytes) or in the quantity of haemoglobin in the blood.

Catheter: A tubular instrument to allow the passage of fluid from, or into, a body cavity.

Corticosterone: A steroid hormone of the adrenal cortex, which mediates responses to stress in addition to regulating protein and carbohydrate metabolism.

Hypovolaemia: Abnormally decreased volume of circulating fluid (plasma) in the body.

Lock solution: A solution used to maintain catheter patency. There are numerous types of lock solution, however heparinsed saline (0.9% saline with 20-500 IU/ml) is most commony used.

Sepsis: The presence in the blood or other tissues of pathogenic micro-organisms or their toxins; the condition associated with such presence.

Thromophlebitis: Inflammation of a vein associated with the formation of a thrombus (an aggregation of blood factors, primarily platelets, fibrin and cellular elements, frequently causing vascular obstruction at the point of its formation).

Vascular access port: A permanent device implanted under the skin, consisting of a vascular catheter attached to a small reservoir, so that blood can be repeatedly drawn on a regular basis. Because the port does not exit through the animal's skin, there is little concern about the animal disturbing the port, thereby obviating the need for a jacket or other protective apparatus. Because there is no chronic exit site wound, infection risks associated with ports are considerably lower than with external catheters.

Vascular puncture: Puncture of a blood vessel.

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