Practical capillary microsampling – avoiding the pitfalls

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Abstract

The introduction of capillary microsampling (CMS) has the 3Rs benefit of refining blood collection whilst reducing the number of animals required for toxicokinetic assessment in rodent toxicology studies. There is also a scientific benefit through directly correlating study findings with systemic exposure. Understandably there is an industry drive towards the use of CMS and consequently positive aspects are promoted in scientific literature. However, capillary sampling has some practical challenges. CMS is offered by Envigo and this article gives advice on: how to collect capillary blood whilst avoiding the introduction of bubbles; how to centrifuge capillaries whilst avoiding sample loss through leakage or capillary breakage; and what to do in the event of difficulties in end to end transfer.

Vasodilation through warming

It is common practice to place rodents into a warming chamber immediately before blood sampling to achieve vasodilation and ensure good blood flow for sample collection. Small volumes are collected using microsampling and it is necessary to have a good blood flow to harvest samples effectively. However, there is a welfare advantage that the duration of warming can be reduced compared with conventional sampling and in the case of larger rodents the use of a warming chamber may not be necessary.

Restraint during sampling

A tube restrainer is typically used for conventional sampling. Due to the smaller volume, it is generally quicker to collect microsamples compared with conventional sampling. Manual restraint may further reduce the period of restraint. Experience has shown that manual restraint results in more effective blood collection, particularly at the start of the study when animals are unfamiliar with tube restraint.

Venepuncture/ harvesting

Blood is typically accessed via the tail vein in rodents. Initially, a needle was inserted into the vein and the blood is then collected from the surface of the tail. This method sometimes resulted in blood flow stopping before the full sample is collected and also increases the likelihood of any test item on the surface of the tail contaminating the sample. Therefore, suggested practice is to leave the needle in the vein whilst blood is collected to ensure continued blood flow and reduce the incidence of repeat needle sticks to finish sampling.

Blood is drawn up the glass tube by capillary action and sometimes the rate of uptake into the capillary exceeds the rate of blood flow out of the vein. This results in the introduction of bubbles in the capillary blood. These bubbles are eradicated from the capillary during centrifugation. However, the presence of bubbles reduces the volume of blood in the capillary and consequently reduces the volume of plasma/serum available.

The two photographs below show the sample capillaries before and after centrifugation and demonstrate the reduced plasma yield in the top capillary which contains bubbles.

Figure 1: Capillaries with and without bubbles of air

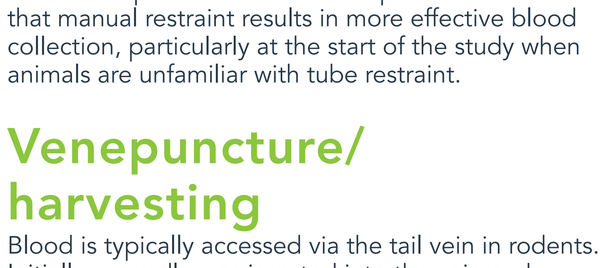
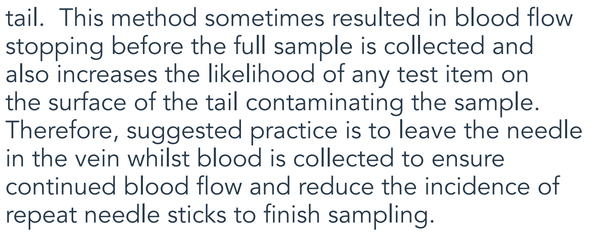


Figure 2: Capillaries from above following centrifugation

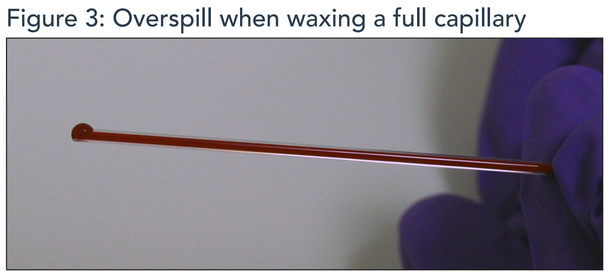


The ideal method is to wait for a drop of blood to form at the end of the needle before starting collection. Blood is instinctively harvested as soon as it emerges from the needle but allowing time for a drop to form creates a reservoir which reduces introduction of air. Additionally the drop of blood at the end of needle should be expanding when the capillary tube is introduced, this shows continued blood flow and further reduces the potential for bubbles.

Wax sealing of capillaries

Once blood has been harvested, one end of the capillary is sealed with wax to keep the contents in the capillary during centrifugation. When the capillary is inserted into the wax block the contents will be displaced. If the capillary is completely filled with blood then some blood will spill over the end wasting the blood and potentially leading to contamination. Leaving sufficient dead space when filling capillaries reduces the volume of blood taken and allows room for displacement due to the wax.

Figure 3: Overspill when waxing a full capillary



Occasionally, when the end of the capillary is withdrawn from the wax, the wax remains attached to the plate rather than inside the capillary. This is avoided by pushing the capillary to the bottom of the wax plate and twisting the capillary before it is withdrawn from the wax. There have been instances of the wax becoming detached from the capillary during centrifugation leading to loss of the sample. Lower centrifuge speeds (discussed later) have reduced this occurrence, however, plugging the same end of the capillary with wax twice helps avoid sample loss.

Ensure wax remains at ambient temperature during use as when too warm it may become soft and fail to seal the capillary during centrifugation. This applies particularly if the work surface used for blood sampling is the top surface of the warming chamber.

Centrifugation of samples

After one end of the capillary has been sealed with wax the plasma/serum is separated by centrifugation. The centrifugation duration and speed are lower for plasma than serum. Therefore, at Envigo, they are processed slightly differently.

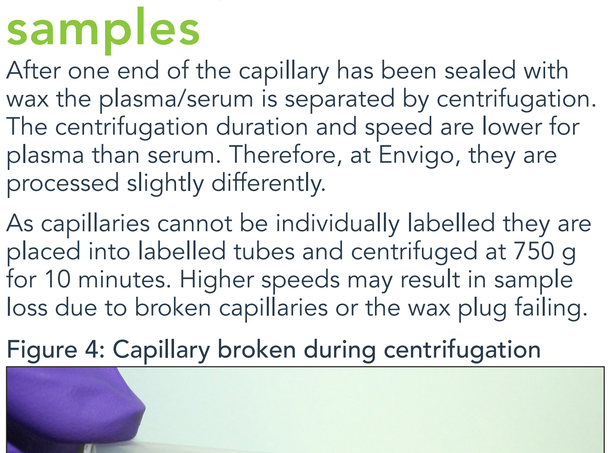
As capillaries cannot be individually labelled they are placed into labelled tubes and centrifuged at 750 g for 10 minutes. Higher speeds may result in sample loss due to broken capillaries or the wax plug failing.

Figure 4: Capillary broken during centrifugation



Separation of serum requires samples to be centrifuged at a higher speed and longer duration than plasma which increases the possibility of sample loss through leakage and snapped capillaries. Therefore, a haematocrit rotor may be used to separate serum. The wax end of the capillary pushes against a flat surface and the length of the capillary is supported to reduce the potential for snapping. The capillaries cannot be individually identified using this technique, however, each capillary bay on the rotor is numbered so a record must be kept of the capillary identity in each bay.

Figure 5: Haematocrit rotor

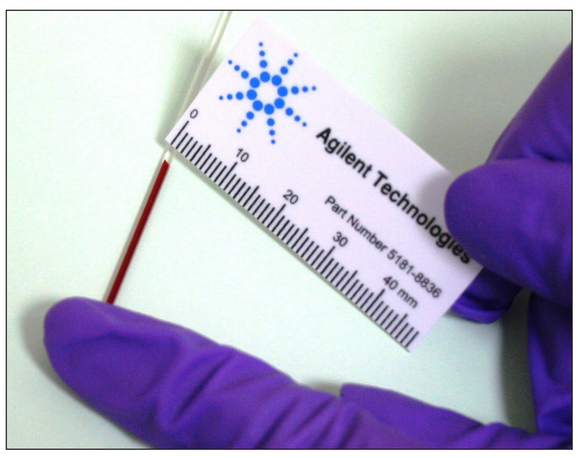


Concern was raised about the potential for cross contamination as a result of spinning open end capillaries together. However, a trial was performed centrifuging a mixture of blank capillaries intermixed with capillaries filled with a dye. The results showed there was no contamination of the dye between capillaries.

Collection of plasma/ serum

Following separation, the capillary is scored in the region containing plasma/serum close to the interface with the blood. The capillary is then snapped and the separated plasma/serum is either expelled into a suitable vessel or collected into a small volume end to end capillary. When scoring the capillary measures should be taken to ensure a clean cut. This would be a single clean cut perpendicular to the capillary. The glass cutters should be renewed regularly.

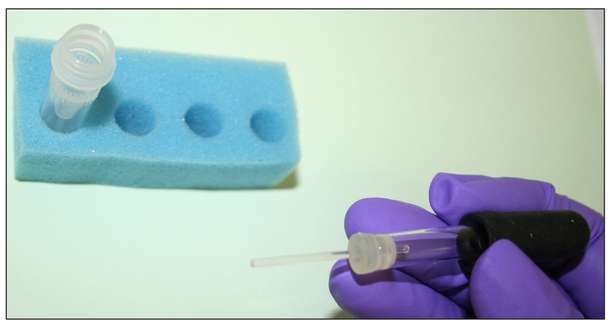
Figure 6: Scoring the capillary



The analytical sample can be collected by expelling the capillary contents into a tube or by end to end transfer to a smaller capillary.

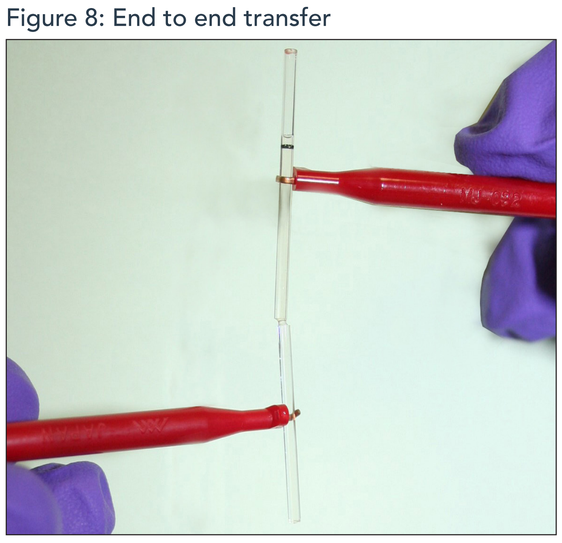
When expelling the plasma/serum into a tube, use a small volume tube (not exceeding 0.5 mL) with a conical bottom. This minimises dead space and improves access to the contents. Tap the bottom of the tube on the bench to ensure all the contents are collated in the conical bottom of the tube before freezing. The capillary contents can be blown out using a microcapillary pipette (Microcaps®) bulb assembly or trimmed pipette.

Figure 7: Expelling plasma using a Microcap®



Alternatively the plasma/serum is transferred to a small volume end to end capillary. The contents usually flow quickly and easily between the two tubes. If content flow is slow then make every effort to avoid the introduction of air bubbles into the analytical sample. Due to the small volumes involved (8 µL), the sample in the end to capillary is typically diluted before assay therefore any inaccuracy introduced through bubbles in the sample is substantially amplified. Flow should not be encouraged between the two tubes by tapping the ends together as this may introduce bubbles into the analytical sample. If the plasma fails to transfer altogether then check the break is clean. It maybe necessary to make another score/snap in the capillary if volumes allow. Alternatively, blow the contents out using the technique above. If blowing out the contents, make it very clear that this has been done as the bioanalytical staff are expecting an accurate, measured volume inside a small capillary.

Figure 8: End to end transfer



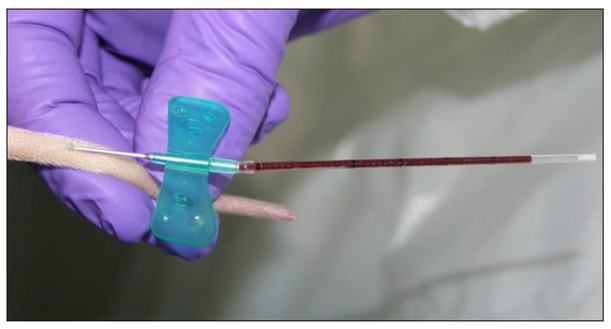
In some instances it may not be possible to completely fill the end to end capillary with plasma/ serum, particularly if there were air pockets in the blood sample. If the end to end capillary is only partially filled, measure the total length of the capillary and then measure the length occupied by the contents. This will allow a reasonable estimation of the volume contents and again ensure that Bioanalysis staff are aware. For example an 8 µL end to end capillary is 24 mm in length, if the plasma occupies 21 mm, the volume it holds is

$$\frac{21\ \text{mm}}{24\ \text{mm}} \times 8\ \mu\text{L} = 7\ \mu\text{L}.$$

Drummond sampling capillary

Drummond Scientific Company have a range of 75 µL collection capillaries which have a Mylar™ sheath (for safety) an integral wax plug and thixotropic gel (to separate the blood cells and plasma/serum). These capillaries can be mounted in the trimmed line of a butterfly needle to create a closed system for blood sampling which also eliminates the introduction of bubbles into the capillary. Following separation the plasma/serum is accessed by advancing the contents through the capillary using a Wiretrol. Initially the plug is stiff to move, but once dislodged it moves smoothly. An issue encountered following centrifugation at 2000 g is that the RBC flowed past the plug and mixed with the plasma as the contents of the tubes were advanced. Drummond Scientific were made aware and advised the thixotropic gel was designed to migrate at higher speeds (11000 rpm). The gel in the tubes was therefore amended so it migrates at lower speeds.

Figure 9: Sampling using a Drummond plasma separation capillary mounted in a butterfly needle



There are some additional bioanalytical considerations for the Drummond capillary compared with the plain glass capillaries as discussed in the European Bioanalysis Forum Open Symposium 2015. One of these considerations is the assessment of analyte binding to the thixotropic gel.

A microsample is generally defined as a volume of less than 50 µL, whereas the Drummond capillaries exceed this with a volume of 75 µL. Advice from Drummond Scientific for smaller volumes is to part fill the capillaries and stand them up (with the wax plug at the bottom) so the blood flows through the capillary to contact the wax plug to ensure a seal before centrifugation.

Summary

- Capillary microsampling offers significant welfare and scientific benefits.
- Microsampling has less impact on the animal due to lower sampling volumes, reduced period of warming and a shorter duration of restraint.
- Techniques are available to ensure continued blood flow from the tail and prevent repeated needle sticks for the same sample.
- Introduction of bubbles into the capillary blood samples reduces the sample volume and can be avoided.
- Leakage of samples and breakage of capillaries during centrifugation is overcome using lower speeds.
- Different centrifuge conditions are required for the separation of serum.
- Expelling plasma from capillaries is a viable alternative to end to end transfer.
- The Drummond plasma separation capillary™ is a closed system which eliminates the introduction of bubbles and leakage during centrifugation, but has additional bioanalytical considerations due to thixotropic gel.

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