

Refined Methods for Blood Sampling

Blood sampling is one of the most common procedures performed on animals in research. To improve animal welfare and ensure high-quality research results, you need to choose an appropriate sampling method and carry out the procedure correctly.

Whether you're experienced in planning and performing blood sampling or are doing it for the first time, it's important to pause and consider how you will approach the task. Several factors must be taken into account, such as the sample type and volume, the sampling site, the instrument used, and how you will prepare, train and handle the animal before and during the procedure.

Below is information that can guide your choice of method. It is primarily intended for those working with animals housed in research facilities or otherwise kept by humans. However, parts of the information may also be relevant if you work with wild animals in research.

If you already have an ethical approval and discover a way to refine your procedures without negatively affecting animal welfare, contact your Animal Welfare Body to get an assessment of whether they can help you amend your ethical approval.¹

What Blood Volume Do You Need?

The sample volume and the sampling frequency should be based on the scientific purpose of the study and the animal's total blood volume. You should also consider the animal's health status and the combined effect of sample volume and frequency. As a general principle, both should be kept to a minimum.

According to general guidelines in Swedish legislation on laboratory animals (14 ch. 5 § Statens jordbruksverks föreskrifter och allmänna råd (2019:9) om försöksdjur, saknr. L150), no more than 10% of an animal's total blood volume may be taken at a single time. If the maximum volume is taken on multiple occasions, there should be at least 14 days between samplings.

When producing polyclonal antibodies, blood sampling must not occur more frequently than the time it takes for blood volume, haemoglobin levels, and red blood cell count to return to normal. The animal's sensitivity to blood loss should

¹ 5 ch. 6 § SJVFS 2019:9.

be considered when planning such sampling.² We recommend applying the same caution for other types of blood sampling.

Taking too large a sample relative to the animal's blood volume can cause suffering in the form of hypovolemic shock (a condition caused by an abnormally low amount of circulating blood), followed by complications such as anaemia. This is especially important if the animal's health is already affected by the study, in which case the maximum sample volume may need to be adjusted.

More information on general principles for blood sampling

[Blood sampling: General principles | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/blood-sampling-general-principles).

Species specific guidance (mouse, rat, rabbit, hamster, and others)

[Blood sampling | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/blood-sampling)

[Recommendation for blood sampling in laboratory animals, especially small laboratory animals | GV-SOLAS \(gv-solas.de\)](https://gv-solas.de/recommendation-for-blood-sampling-in-laboratory-animals-especially-small-laboratory-animals)

Can You Use Microsampling?

Improved methods and new technologies now allow for smaller sample volumes in a wider range of analyses. Samples of up to 50 µl (microliters) are called microsamples. Microsampling is faster and requires less handling than larger volumes, and can thus contribute to improved animal welfare.

One area where microsampling has proven useful is in assessing drug and chemical exposure in blood, plasma, and serum. In some cases, drug levels and biochemical parameters can be analysed from samples as small as 5–20 µl. In such studies, microsampling can also reduce the number of animals needed and improve research quality, as the small volume allows for multiple samples from the same animal to be taken at different stages of the study.

Microsampling can enhance animal welfare because:

- The procedure results in less blood loss.
- The procedure is less invasive.
- The procedure is quicker and therefore less stressful for the animal, regardless of species.
- The need to warm the animal to increase peripheral blood flow may be significantly reduced.
- Refined sampling techniques can be used.

² 14 ch. 5 § SJVFS 2019:9.

- Refined handling methods can be applied, reducing or eliminating the need for restraint.

More information on microsampling and study design

[Microsampling | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/microsampling)

[Microsampling study designs | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/microsampling-study-designs).

Other Factors to Consider When Choosing a Sampling Method

There are several practical aspects you need to consider when planning your sampling:

- Sampling from a clearly visible, superficial blood vessel reduces the risk of injury and helps ensure high sample quality.
- The size of the needle (length and diameter) is important for controlling the amount of blood drawn. Make sure the needle has a sufficient diameter to allow for quick blood collection, while still being slightly smaller than the vessel's diameter to avoid rupturing the vessel or damaging surrounding tissue. For superficial vessels, short needles should be used.
- Once the sample has been taken, ensure that bleeding stops. You can apply gentle pressure to the puncture site for a few minutes to help stop the bleeding. After arterial sampling, you may need to apply pressure for a longer period.

More information on choosing sampling methods

[Blood sampling | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/blood-sampling)

[Blood sampling | Norecopa \(norecopa.no\)](https://norecopa.no/blood-sampling)

Avoid Reuse of Needles

Single-use (disposable) needles are designed to be used once. Even after the first puncture, the tip is likely to become damaged and dulled. Reusing the needle can cause pain to the animal, as the blunted tip creates larger and more uneven tissue damage. Reuse can also lead to contamination. Therefore, as a standard practice, you should always use single-use needles only once, regardless of how minor you consider the procedure to be.

More information on single use of needles

[Single use of needles | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/single-use-of-needles)

Handling and Training for Blood Sampling

When planning your study, you need to consider how you will handle the animals during blood sampling and whether it is relevant to train the animals prior to sampling.

How animals are handled, trained, and otherwise prepared for blood sampling can have a significant impact on the success of the procedure. An unsuccessful sampling attempt often results in reduced animal welfare, since the animal may experience stress and additional needle sticks.

Calm and unstressed animals are generally easier to sample from. This is partly because they remain more still, which improves accuracy, and partly because blood vessels tend to constrict under stress. One way to dilate the vessels and improve accuracy is to warm the animal, for example in a warming cabinet, by immersing the tail in warm water, or similar methods. Dilating the vessels in this way can reduce the number of punctures needed. However, be aware that sudden warming and unfamiliar environments may increase the animal's stress levels.

Keep in mind:

- Always habituate animals to being handled by humans.
- Always handle animals calmly and gently.
- It is preferable to avoid restraining the animals completely; instead, hold only the body part from which the sample is taken.
- Avoid using restraining devices.
- If you need to use a restraining device, train the animals to become habituated to it.
- Depending on the species and the scientific purpose of the study, consider offering a reward after each sampling.

All personnel involved in animal experimentation must be trained and competent in their work.³ If you are unsure whether you can perform blood sampling in the least stressful way possible, there is often experienced staff available who can take the samples for you or offer guidance and advice.

Don't hesitate to ask for help – remember that any stress experienced by the animal can affect the quality of the sample and, consequently, your results and the overall quality of your research.

³ 6 ch. 2–3 §§ SJVFS 2019:9.

More information on handling and training animals for procedures like blood sampling

[Handling and training of mice and rats for low stress procedures | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk).

Assess the Suitability of Your Method

You can use the following criteria to assess whether the blood sampling method you plan to use is appropriate – both from an animal welfare perspective and to ensure high-quality samples:

- You keep sample volumes and the number of samples to a minimum.
- You use microsampling when possible.
- You habituate animals to human handling and handle them as calmly and gently as possible.
- You train animals for blood sampling when relevant.
- The blood vessel you plan to sample from is easily accessible and clearly visible.
- You can control the amount of blood drawn during the procedure.
- You can quickly and easily stop further blood loss after sampling.
- There is no risk of damage to surrounding tissue during or after the procedure.