Tierärztliche Vereinigung für Tierschutz e.V.

GV-SOLAS
Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

Specialist information

from the Committee for Animal Welfare Officers (GV-SOLAS) and Working Group 4 in the TVT

Recommendation for blood sampling in laboratory animals, especially small laboratory animals

Status July 2017

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1. Preliminary remark

This recommendation was compiled for persons submitting applications to conduct animal experiments, for animal welfare officers and for authorities. It is based on the TVT leaflet drawn up by Dr. Werner Nicklas in 1995 entitled "*Hinweise zur Blutentnahme bei kleinen Versuchstieren"* (Notes to blood sampling in laboratory animals) and is intended to serve as a **guide** for humane animal work and to standardize the procedures applied today. **The values given in the tables are recommendations, from which it is possible to deviate if required by the experiment and provided permission to deviate has been obtained.** It is the responsibility of each and every person to keep abreast of the latest knowledge on the optimum procedure to be used for a given technique in a given species.

The recommendation is divided into two sections. The first section discusses the fundamental technical issues of blood sampling, while the second section contains tables on suitable methods of blood sampling and blood volumes.

2. Basic principles of blood sampling

- Based on the physiology of the animal, no more blood may be taken no more frequently than is necessary to achieve the objective of the experiment.
- The sampling site can have an influence on the clinical blood values.
- If blood is taken too quickly, the vessel may collapse.
- The more routinely blood sampling is performed, the better the quality of the blood taken (1) and the less stressful the sampling procedure will be to the animal.
- The diameter and length of the cannula is also important. A long cannula can lead to coagulation already within the needle and hence stop the blood flow. The diameter should be as wide as possible to ensure a rapid blood flow. The use of 20G cannulas has not led to greater tissue damage in mouse and rat than the use of 25G cannulas (2).
- If the blood is taken from a vessel which the person concerned has never used before for blood sampling, it is advisable to isolate the vessel in a dead animal first in order to become familiar with its location. Taking blood from a live animal must initially be performed under expert supervision.
- Unlike in the cat, dog and pig, the fasting of rodents and rabbits before blood samples are taken is counterproductive.

The blood sampling procedure is dependent on various factors, which have to be considered beforehand:

- **Desired quality of blood?**

  sterile – non-sterile

  arterial – venous – mixed blood

  contaminations (decapitation, tail clip, puncture of lingual vein)

  haemolysis

  time lag between feed intake and blood sampling

  (no fasting in rodents and rabbits)

- **Sampling frequency?**

  once – repeated

  time lag between blood sampling procedures

- **Final blood sampling?**
- **Desired volume of blood?**

The procedure in blood sampling depends on the factors listed above. For each specific instance, more or less well-suited procedures exist.

Frequent and gentle handling are very important so that the animals become accustomed to the hand and are prepared for the blood sampling. The use of rewards can be very useful. These measures can help substantially to reduce the level of anxiety and hence the stress on the animals.

In principle, blood sampling techniques should be selected that expose the animal to the least possible stress. Observance of these points has a positive effect both on the animal (less stress) and on the quality of the blood (less stress-induced influence on blood parameters).

With some blood sampling techniques, anaesthesia is absolutely essential for reasons of animal welfare, and this can likewise result in corresponding changes among the control animals. It must be borne in mind that the anaesthetic can also influence circulatory and blood parameters (for example, injection anaesthesia often leads to vasoconstriction).

3. Locations for blood sampling (see also Table 2)

Puncture of ear vein. In the rabbit (marginal ear vein) and the guinea pig, it is usually only possible to obtain small volumes of blood by puncturing the ear vein. It is often sufficient to compress the vein by applying pressure with the fingers. In other small laboratory animals, these blood vessels do not play any role in blood sampling. Anaesthesia is not necessary to puncture ear veins. Cutting into the vein is not humane.

Puncture of central ear artery. Up to 30 ml of blood can be obtained from the ear artery of the rabbit – depending on bodyweight of course – without the need for anaesthesia and without exposing the animal to any substantial stress. To this end, the animal must be restrained so that it cannot injure itself during blood sampling*. Slight hyperaemia at the tip of the ear (see below) is already sufficient to let the artery swell so that it can be securely punctured with a cannula. The use a butterfly cannula is recommended. A disadvantage of taking blood from an artery is the high risk of haematoma or secondary bleeding. After obtaining the blood sample, therefore, the punctured artery must be compressed at or proximal to the puncture site for a sufficient period of time (in some cases up to 5 minutes) to be sure of stopping the blood flow. It is advisable to check after 5 to 10 minutes.

The animal should be restrained either in keeping with animal welfare by an assistant (both forearms on the back of the rabbit, abdomen of the assistant presses the pelvis of the rabbit onto a non-slip underlay) or using a cut-off trouser leg with a Velcro fastener at one end (rabbits like to enter, and the fastener is then fixed in the neck region so the head peeps out and all four limbs remain in the trouser leg, then turn over the hind legs).

Pyrogen boxes are less suitable, because there is a risk of injury in the event of any sudden movement.

Puncture of retrobulbar venous plexus. In the mouse, hamster and rat, relatively large volumes of blood can be obtained quickly using this method. Under brief general anaesthesia, e.g. with isoflurane, the neck veins of small rodents are compressed by gripping the scruff of the neck. Guide a capillary that is not broken off (check with fingertip) and as a rule not heparinized (risk of secondary bleeding) through the conjunctiva under pressure and with slightly rotating movements in the inner corner of the eye in the direction of the opposite mandibular joint and puncture the venous plexus.

If blood does not immediately appear, withdraw the capillary very slightly. In the mouse and hamster, capillaries with an external diameter of 0.8 mm should be used, while in rats capillaries with an external diameter of 0.9 mm are acceptable. Doubling of the diameter results in a traumatized area four times greater. Before removing the capillary, loosen the grip on the scruff of the neck to keep bleeding into the tissue to a minimum. **Repeat blood sampling from an eye after two weeks at the earliest!**

Some studies on the impact of puncturing the retrobulbar venous plexus in the rat have found that the well-being of the animal is not notably compromised if it is performed properly (3, 4), although changes in activity have been demonstrated in rats within the first 20 hours (5).

For rats, this blood sampling technique is only recommended up to an age of **not more than six months**, because the fatty tissue in the region of the venous plexus can clog the capillary in older rats.

Puncture of the facial vein (mandibular region). This method is **only** to be used in mice. As with other techniques, it also requires sufficient training. No restraining apparatus and also no anaesthesia are needed. It must be borne in mind that there are strain-specific differences for the puncture site. Using a 4–5.5 mm lancet, the puncture is performed 3–4 mm dorsocaudal to the whorl of hair at the mandible; depending on bodyweight, up to a maximum of 300 µl of blood (in large mice) can be obtained in this way. After blood collection, compress the puncture site for up to a minute to avoid a haematoma. It is important for the success of this method that the animal is properly restrained (stasis of the vein by pulling back the facial skin – “face lifting”) and the penetration depth is correct (6), which is ensured when the above-mentioned lancets are used. Do not maintain stasis for too long, since this could lead to strain-specific death.
https://medipoint.com/for-use-on-mice/

Puncture of sublingual vein. The technique as it was originally described (7) has been refined (8) and is more complex, requiring the involvement of two people and the use of anaesthesia. The puncture can be used in hamsters, guinea pigs and rats. The first person holds the animal by the scruff of the neck to produce a slight stasis of venous reflux from the head and turns it on its back. The second person holds the tongue with thumb and index finger. The two sublingual veins can be identified alongside the median line and can be punctured using a 23G needle. The animal is turned back to a prone position and held over a collecting vessel into which the blood can drip. The animal must be held as horizontally as possible to avoid accidental aspiration of blood. The grip on the scruff of the neck must then be relaxed and the bleeding can be stopped using a ferric chloride solution (20–30%) and cotton swabs.

If feed refusal lasting more than a day occurs, as happens in exceptional cases, the animal must be euthanized (9).

Puncture of jugular vein. Blood samples are taken from the jugular vein under anaesthesia in laboratory animals in which the vein has to be dissected (e.g. hamster, guide pig, ferret). In larger animal species (e.g. bovine animal, sheep, goat, pig, horse, dog or cat) it is sufficient to generate stasis and take the blood directly through the skin.

Puncture of venous angle. Mouse, rat, gerbil and guinea pig are placed in supine **on the bench under anaesthesia**, the front legs are extended caudally alongside the body and the hind legs restrained to avoid a change of position during blood sampling. In his position, the venous angle - where the internal jugular vein meets the external jugular vein - is located below the clavicle (collar bone). Puncture the vein in the cranial region of the neck in the direction of the contralateral knee and below the clavicle. Caudal to the clavicle, the venous angle may also be punctured vertically. With this method, the largest possible volume of blood is obtainable quickly and under sterile conditions. With **practised** use of the procedure, haematoma and injuries to the lung can be largely excluded. **Unpractised** use of the procedure can result in injuries to the lung and death (10, 11, 12).

Puncture of the tail vein. In mice and rats, small quantities of blood (a few drops) can be obtained from the tail vein by puncturing it with a needle. It is often sufficient to create slight stasis of the blood vessels by applying finger pressure to the base of the tail (13). To obtain larger volumes (200 µl in mouse; 1 ml in rat) the two collateral veins on the right and the left in the mid third of the tail are suitable. After immobilization of the animal in a restrainer, hyperaemia should be induced in the tail (see 3.). The index finger is placed on the mid third of the tail. The cannula (22-23G, cone cut off) is inserted into the vein in the bend in a flat angle (almost parallel) in in the direction of the tail base (14). The capillary action of the needle can be exploited by then attaching haematocrit tubes, but the blood can equally be collected in tubes. What is essential for successful blood sampling is a calm atmosphere, because stress causes vasoconstriction.

The tail vein is also very suitable for blood sampling in certain primates (e.g. marmosets), ferrets and bovine animals.

Amputation of tail tip and incision of tail vein. Amputating more than 5 mm of the tail tip in the mouse and rat is not acceptable. Appropriate haemostasis is required. This blood sampling technique should not be used in older animals.

Repeated incision of the tail vein should be avoided (15), because this can lead to granuloma formation in rats.

Puncture of the saphenous vein. Blood sampling from the saphenous vein has been successfully used in the mouse, rat, hamster, gerbil, guinea pig, rabbit, mink, ferret, dog, cat and pig (16, 17). Pressure is applied to the vein of the restrained animal above the knee joint. Mice are readily restrained in a Falcon tube, the tip of which has been cut off for air intake.

It is the method of choice for the guinea pig, although the vein is often not visible or palpable in this species even when pressure is applied. A puncture made directly lateral to the Achilles tendon in the mid third of the lower leg should prove reliable if made at an angle of less than 45°.

Up to 5% of total blood volume can be obtained using this method. The vein is more visible if the area is shaved and disinfected with alcohol (18).

Mouse: https://norecopa.no/norina/blood-collection-in-mice-using-the-saphenous-vein-an-alternative-to-retro-orbital-collection

Puncture of aorta, vena cava and other large blood vessels. In the pig, blood sampling from the cranial vena cava or the brachiocephalic vein is the method of choice when larger volumes of blood (> 5 ml) are to be collected. In the dog and cat, blood sampling from the cephalic antebrachial vein or the saphenous vein is a good option. In the dog, blood may also be taken from the jugular vein.

In smaller animal species, blood sampling from large blood vessels is considered mainly for terminal blood collection. In small rodents in particular, such as the rat and the mouse, exsanguination by puncturing the abdominal aorta or the caudal vena cava (ideally at the level of the right kidney) are very suitable methods for obtaining a high yield. Under deep anaesthesia, the abdominal cavity is opened, and the vessel punctured (23G) or opened under visual supervision.

Taking blood from the femoral vein is less common. But it is possible in various animal species (e.g. rat, ferret, monkey), in which case, the procedure is performed in the anesthetized supine animal. In smaller animals, it is not customary to puncture this blood vessel.

Cardiac puncture (terminal procedure only). For this procedure, the laboratory animals are anaesthetized and restrained in a supine or lateral position. The cannula is inserted through the skin next to the sternum vertically to the surface of the body from the lateral or ventral direction until the pulsation of the heart is palpable and blood leaks through the cannula. A favourable puncture site is the area in which the apical impulse is most clearly palpable. A puncture in the median line behind the xiphoid (in supine position) is similarly good. It is important to withdraw the blood slowly, because an excessively negative pressure will lead to cardiac collapse, which will not allow any further blood to be collected. Since cardiac puncture should only be performed in terminal experiments (19), the heart may also be punctured in the opened rib cage (especially in rodents).

4. Generating hyperaemia or dilatation of blood vessels

When taking blood from the blood vessels of the ear (especially the artery) or from the tail vein (in mice and rats) vasodilatation is usually helpful before puncturing the vessel. In rabbits and pigs, gentle tapping of the ear with the fingertips is often sufficient; in rabbits, good hyperaemia can also be produced by plucking out hairs over the puncture site. The same effect can also be achieved by warming the ear, e.g. with a heat lamp. Local application of heat is also very suitable for dilatation of the tail veins in rodents (brief immersion of the tail in water at about 45°C; while keeping the tip of the tail out of the water because it is especially sensitive). Warming of the whole body as recommended in some publications (with infrared lamp) must be applied with caution, because it can lead to a rise in core body temperature to more than 42°C.

The use of xylene and similar substances that produce hyperaemia by irritating the skin (skin necrosis) should be prohibited for reasons of animal welfare. It is also advisable not to use

ointments that produce hyperaemia, because they dry out the skin and can be absorbed orally as a result of grooming.

5. Sampling small quantities of blood (see data in Table) (some drops, a few ml in large animals)

Smaller volumes are obtained in rat and mouse by puncture of the tail vein (a few drops) and in rabbit and guinea pig by puncture of the marginal ear vein or saphenous vein. In rabbits, the use of small-lumen butterfly needles can be advantageous, because they offer greater flexibility with regard to unintended movements. In chickens, a small quantity of blood can be taken from the comb or the wing vein.

6. Taking single samples of a larger volume of blood

Larger volumes of blood in animals up to about rabbit size are obtained by cardiac puncture (terminal procedures only) and in large animal species usually by the puncture of large blood vessels (e.g. jugular vein, ear vein and, in rabbits, also ear artery). If an animal is intended to survive, the maximum recommended volume of blood to be taken must not be exceeded, so as to avoid exposing the animal to stress. This volume of blood depends on many factors, such as animal species, breed, weight, sex, age and the nutritional and health status. In relatively large animals of one species, the blood volume relative to bodyweight is smaller than that of smaller animals of the same species. It is difficult to determine the blood volume precisely, so rough estimates are usually made. Criteria for estimating blood volume in some animal species are listed in Table 1.

As a rule, taking up to 10% of blood volume is tolerated without any noticeable side effects. But when a volume of 10% or more is taken, compensatory mechanisms are already set in motion (15). To avoid side effects (fall in blood pressure, necrosis of gastric mucosa, increase in concentrations of adrenal or pituitary hormones as indicators of stress and a reduction of the haemoglobin concentration in the blood) (20), the volume of blood taken should be substituted with sterile isotonic physiological saline at body temperature.

NOTE: in practice, the incorrect rule is often applied to set the blood volume at 10% of bodyweight and to take 10% of this volume. The resulting maximum sample quantity of 1% of bodyweight gives excessively high values, because the real blood volume in mammals amounts to about 6–8% of bodyweight.

7. Repeated blood sampling

With repeated blood sampling, especially in cases where small volumes are taken at very short intervals, the use of indwelling catheters or fully implantable catheter systems makes sense and is less stressful for the animals than repeated puncture of the vessels (for details see 4, 15, 21, 22). It must be borne in mind that, despite the use of such systems, the corticoid concentration is much higher with frequent blood sampling than when fewer samples are taken over the same period (23). An alternative method for pharmacokinetic studies is peritoneal microdialysis (24).

The recovery phase after blood sampling depends on the volume of blood taken. When the maximum acceptable quantity of 10% of estimated blood volume is taken, a recovery phase

of at least 2 weeks is necessary in principle. In the case of relatively frequent blood sampling, the weekly quantity taken should not exceed 7.5% of blood volume. A rule of thumb is that not more than 1% of blood volume must be taken from a laboratory animal per day. Generally, (in a healthy, adult, normally fed animal) this corresponds to about 0.6 ml/kg bodyweight per day.

8. Exsanguination

For exsanguination, an animal as a basic principle must be anaesthetized. It dies under anaesthesia as a result of blood loss (the onset of death must be monitored particularly in animals that are not dissected!). Exception: humane decapitation of rat and mouse.

Smaller animals up to about rat size can also be well exsanguinated by puncture of the large abdominal arteries or the heart of the dissected animal. Larger animal species such as rabbit, dog and cat are usually exsanguinated by cardiac puncture or from an exposed carotid.

9. Workup of the blood

For animal welfare reasons, unnecessary repeated blood collections must be avoided. Before blood sampling, therefore, it is necessary to make sure further workup of the blood is performed in line with standard practice.

10. Stress from blood sampling

If the recommended maximum quantities and minimum intervals between blood sampling procedures are observed, a mild to moderate stress (duration < 1 days) can be generally assumed as a consequence of the blood sampling. If the stress for the animal in kinetic studies is too much from the animal welfare standpoint, the use of indwelling catheters can minimize the stress.

The entire problem of the biological effects caused by blood loss following single or repeated blood sampling is described in detail by McGuill & Rowan (1989) (20). The authors also discuss the suitability of different blood sampling techniques in rat, mouse and rabbit. Blood sampling techniques are described in detail by Grice (1964), Herbert & Kristensen (1986) and Iwarsson et al. (1994) (25 – 27).

Table 1: Average blood volume in different animal species

Species	Total volume		Haematocrit (%)	Estimated absolute blood volume at indicated weight (ml)	Example of blood sampling usage[2] (ml) at indicated bodyweight		
	ml/kg b.w.	% b.w.			once[3] (max. 10%)	daily[4] (1%)	terminal[5]
Mouse (25 g)	74 (70 - 80)	**7.4**	42 (33 - 50)	**1.7**	**0.17**	**0.02**	**0.7 – 1.0**
Rat (300 g)	64 (50 - 70)	**6.4**	46 (40 - 61)	**19**	**1.9**	**0.2**	**10**
Guinea pig (400 g)	75 (65 - 90)	**7.5**	44 (37 - 50)	**30**	**3.0**	**0.3**	**15**
Golden hamster (100 g)	78 (65 - 80)	**7.8**	51 (39 - 59)	**7.8**	**0.7**	**0.07**	**3.5**
Gerbil (100 g)	67 (60 - 85)	**6.7**	48 (40 - 52)	**6.7**	**0.7**	**0.07**	**3.3**
Ferret (800 g)	75 (60 – 80)	**7.5**	45 (38 - 54)	**60**	**6.0**	**0.6**	**30**
Rabbit (3.2 kg)	56 (45 - 70)	**5.6**	41 (31 - 50)	**180**	**18**	**1.8**	**90**
Dog (15 kg)	86 (79 - 90)	**8.6**	50 (42 - 58)	**1300**	**130**	**13**	**650**
Cat (3 kg)	56 (47 - 66)	**5.6**	38 (30 - 45)	**168**	**17**	**1.7**	**84**
Chicken (1,1 kg)	65 (60 - 90)	**6.5**	34 (25 - 45)	**71**	**7.1**	**0.7**	**36**
Marmoset (350 g)	70 (58 – 82)	**7.1**	45 (37 – 52)	**25**	**2.5**	**0.25**	**12**
Rhesus monkey (9 kg)	54 (44 - 67)	**5.4**	41 (33 - 50)	**480**	**48**	**4.8**	**240**
Mini-pig (20 kg)	65 (61 - 68)	**6.5**	39 (30 - 50)	**1300**	**130**	**13**	**650**
Sheep (50 kg)	66 (55 - 80)	**6.6**	32 (26 - 37)	**3300**	**330**	**33**	**1650**
Goat (40 kg)	70 (57 - 90)	**7.0**	33 (26 - 37)	**2800**	**280**	**28**	**1400**
Bovine (400 kg)	57 (52 - 61)	**5.7**	40 (33 - 50)	**22800**	**2280**	**228**	**11400**
Horse (300 kg)	75 (56 - 118)	**7.5**	33 (26 - 42)	**22500**	**2250**	**225**	**11250**

[1] Differences in terms of breed, strain and age are possible; the percentage is lower in obese animals than in those of normal weight.
[2] These blood sample volumes apply to healthy, adult animals. Animals after experimental manipulation or sick, old or stressed animals may not tolerate the same blood sample volumes.
[3] subsequent recovery phase of at least 2–3 weeks
[4] daily blood sample volume over max. 2 weeks, subsequent recovery phase of at least 2–3 weeks
[5] under anaesthesia; about 50% of blood sample volume (empirical value)

Table 2: Sites for blood sampling

	Mouse	Rat	Hamster	Gerbil	Guinea pig	Rabbit	Dog, cat	Chicken	Pig	Sheep, goat	Bovine, horse
Ear vein					S	S			S		
Ear artery						L					
Retrobulbar venous plexus[1]	L	(L)	L	S							
Facial vein	L										
Comb (chicken)								S			
Jugular vein			L		(E) [1]	(E) [1]	L		S, L	S, L	S, L
Venous angle	L[1]	L[1]	L[1]	L	L[1]						
Cephalic antebrachial vein							S, L				
Saphenous vein	S	S	S	S	S	S	S, L		S, L		
Tail vein	S, L	S, L		S							S (bovine)
Vena cava[2]	E	E	E		E	E			S[3], L[3]		
Sublingual vein		S	S		S						
Wing vein (ulnar vein)							S	S			
Aorta, large abdom. arteries[4]	E	E	E		E	E					
Heart	E	E	E		E	E	E	E	E	E	E

Key:
S = suitable for small blood volumes; L = suitable for larger blood volumes; ( ) = limited suitability; E = suitable for exsanguination

[1] only under anaesthesia; [2] under anaesthesia with opened abdominal cavity or opened rib cage (except in pig), [3] Cranial or brachiocephalic vena cava;
[4] under anaesthesia with opened abdominal cavity or opened rib cage [5] only terminal under anaesthesia

Table 3: Mouse

Sampling volume and frequency	Sampling site	Special requirements	Estimated level and duration of stress
1. **Small volume** (0.02 – 0.04 ml)	a) Tail vein b) Amputation of tail tip c) Retrobulbar d) Facial vein e) Saphenous vein	b) only up to weaning age c) anaesthesia, (e.g. isoflurane), glass capillary with 0.8 mm external diameter, heparinization unnecessary)	a) mild, < 1 day b-e) mild – moderate, < 1 day
2. **Single maximum** volume **(see** Table **1)** Recovery phase: 2 weeks	a) Retrobulbar b) Tail vein c) Venous angle d) Facial vein	a) as described under 1c) b) cannula: 24 - 26 G c) anaesthesia; cannula: 23 - 25 G	mild – moderate, < 1 day
3. **Repeated sampling (see** Table **1)** When the maximum volume of blood to be taken is reached Recovery phase: 2 weeks	Repeat sampling within 24 h: tail veins daily and weekly sampling: a) Tail veins b) Facial vein c) Saphenous vein d) Retrobulbar	With all types of blood sampling select alternating sampling sites. d) anaesthesia, per not more than two sampling procedures at an interval of 14 days	mild – moderate, < 1 day
4. **Terminal sampling (exsanguination)**	a) Cardiac b) Aorta, Vena cava c) Decapitation	a, b) anaesthesia c) also possible without anaesthesia	a-c) mild

Table 4: Hamster and gerbil

In the case of repeated manipulation, especially with golden hamsters, care must be taken to ensure that handling causes the least possible stress, because otherwise the animals become increasingly aggressive. In smaller hamster species (e.g. Chinese dwarf hamsters) few of the specified methods are suitable for taking blood. In the case of the gerbil/Mongolian jird (*Meriones unguiculatus)*, particular caution is called for with **any** manipulation involving the tail, because otherwise skin defects may occur. Unless otherwise indicated, the methods specified below apply to both species (for cannula size, see mouse).

Sampling volume and frequency	Sampling site	Special requirements	Estimated level and duration of stress
1. **Small volume** (0.02 – 0.04 ml)	a) Saphenous vein b) Tail vein in the gerbil	Anaesthesia, (e.g. isoflurane)	mild, < 1 day
2. **Single maximum** volume **(see** Table **1)** Recovery phase: 2 weeks	a) Retrobulbar b) Saphenous vein c) Tail vein in the gerbil d) Puncture of venous angle	a, d) Anaesthesia (e.g. isoflurane) a) Capillary with 0.8 mm external diameter	mild – moderate, < 1 day
3. **Repeated sampling (see** Table **1)** When the maximum volume of blood to be taken is reached Recovery phase: 2 weeks	a) Indwelling catheter in jugular vein b) Cannulation of the lateral tail vein in the gerbil	a) Protected catheterization: polyethylene, diameter 0.2 mm b) Use restraining tube	a) mild – moderate; depending on duration and frequency b) mild < 1 day
4. **Terminal sampling (exsanguination)**	a) Cardiac b) Vena cava c) Aorta	Anaesthesia	mild

Table 5: Rat

Sampling volume and frequency	Sampling site	Special requirements	Estimated level and duration of stress
1. **Small volume** (0.02 – 0.04 ml)	a) Tail vein b) Amputation of tail tip c) Saphenous vein	b) Cannula: 23G - 26G c) Only up to weaning age	a) mild, < 1 day b-c) mild – moderate, < 1 day
2. **Single maximum** volume **(see** Table **1)** Recovery phase: 2 weeks	a) Tail vein b) Retrobulbar c) Venous angle	a) Cannula: 23G - 26G b) Anaesthesia (e.g. isoflurane), capillary up to 0.9 mm external diameter) c) Anaesthesia; cannula: 21G - 25G	a) mild, < 1 day b-c) mild – moderate, < 1 day
3. **Repeated sampling (see** Table **1)** When the maximum volume of blood to be taken is reached Recovery phase: 2 weeks	a) Tail veins b) Indwelling catheter in jugular vein c) Retrobulbar d) Saphenous vein	b) Anaesthesia, polyethylene catheter, external diameter about 0,61 mm, port system c) Anaesthesia, not more than two blood sampling procedures per eye within 14 days	a, b, c, d) mild – moderate
4. **Terminal sampling (exsanguination)**	a) Cardiac b) Aorta c) Vena cava d) Decapitation	Anaesthesia a) Cannula: 20 - 21 G d) Also possible without anaesthesia	a-d) mild

Table 6: Guinea pig

Sampling volume and frequency	Sampling site	Special requirements	Estimated level and duration of stress
1. **Small volume** (0.02 – 0.04 ml)	a) Ear vein b) Saphenous vein		mild, < 1 day
2. **Single maximum** volume **(see** Table **1)** Recovery phase: 2 weeks	a) Jugular vein b) Venous angle	a) Anaesthesia; vessel must be exposed; cannula: 21G – 22G b) Anaesthesia	mild – moderate, < 1 day
3. **Repeated sampling (see** Table **1)** When the maximum volume of blood to be taken is reached Recovery phase: 2 weeks	a) Indwelling catheter in jugular vein b) Ear vein c) Saphenous vein	b) Anaesthesia, polyethylene catheter or subcutaneous port, external diameter 0.6 mm	a) mild – moderate, depending on duration b) mild < 1 day
4. **Terminal sampling (exsanguination)**	a) Cardiac b) Aorta c) Vena cava	Anaesthesia	mild

Table 7: Rabbit

Sampling volume and frequency	Sampling site	Special requirements	Estimated level and duration of stress
1. **Small volume** (1 – 3 ml)	a) Marginal ear vein b) Saphenous vein	Cannula: 20G - 23G	mild, < 1 day
2. **Single maximum** volume **(see** Table **1)** Recovery phase: 2 weeks	Central ear artery	Butterfly: 20G – 22G Pyrogen box; compression of puncture site (up to 5 min)	mild, < 1 day
3. **Repeated sampling (see** Table **1)** When the maximum volume of blood to be taken is reached Recovery phase: 2 weeks	a) Indwelling catheter in jugular vein b) Marginal ear vein c) Saphenous vein	b) Anaesthesia; indwelling catheter b) Cannula 21 – 22 G c) Butterfly: 21 – 22 G pyrogen box; compression of puncture site (up to 5 min.)	a) mild – moderate, < 1 day b-c) mild, < 1 day
4. **Terminal sampling (exsanguination)**	a) Cardiac b) Aorta c) Vena cava	Anaesthesia, cannula 20G	mild

Summary and comparison table for colour coding, dimensions and sizes of cannulas

Pravaz system (size)											1	2	12	14	15	16	17	18	20	21	22				23	
Length according to Pravaz											38	35	32	30	26	26	26	23	22	20	20				20	
Gauge (G)	10	11	12	13	14	15	16	17	18	19	20	21	22			23	24	25	26	27	28	29	30			
Colour according to ISO 6009 or DIN 13095	**brown/ olive**	**yellowish green**	**white-blue**	**purple**	**white-green**	**blue-grey**	**white**	**red-violet**	**pink**	**cream**	**yellow**	**deep green**	**black**	**blue***	*	**deep blue**	**medium purple**	**orange**	**brown**	**medium grey**	**blue-green**	**red**	**yellow**	*	*	*
External diameter (mm) according to ISO/DIN 9626	3.4	3.0	2.7	2.4	2.1	1.8	1.6	1.4	1.2	1.1	0.9	0.8	0.7	0.65*	0.65*	0.6	0.55	0.5	0.45	0.4	0.36	0.33	0.3	0.28*	0.26*	0.25
Commonly used lengths (mm)				80				40	40 50 70		38	30 35 40 50 120	16 30 32	30	26	16 26 30	26	16 24 26	10 16 23	6, 8, 10, 12, 20, 22						

*The DIN/ISO specifications assign the external diameter and colour to the size indicated (Gauge). If no dimension is indicated in Gauge, the figures are not taken from the DIN/ISO standards but are included for the sake of providing an overview

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