

## Technical Report:

# Gingival Vein Puncture: A New Simple Technique for Drug Administration or Blood Sampling in Rats and Mice

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### *Summary*

Blood collection or intravenous injection of substances in rats and mice are necessary for a wide variety of scientific studies. To date, several methods have been developed to access different vessels, according to the different research purpose. However, animal behavioural responses like stress, pain or traumatic injury during some procedures may influence subsequent results. In this technical report we demonstrate the advantages of using the labialis mandibularis vein route that, in addition, seems to prevent unnecessary animal suffering.

### *Introduction*

The intravenous injection of drugs, or blood sampling are among the most frequently used procedures in laboratory animals for pharmacokinetic and pharmacodynamic studies (NC3RS, 2007). For this purpose, several procedures for vascular blood collection have been developed and widely diffused among researchers, each of them with their own characteristic. Since some cause more harm to the animals than others, we must ultimately pay attention to the impact that the chosen procedure may have on a given study. A significant fact is that the most widely used techniques are not fully in accordance with animal welfare principles discussed in guidelines and workshops dedicated to the use of laboratory animals (Morton et al., 1993, 2001; Diehl et al., 2001; Schnell et al., 2002; Lucas et al., 2004).

In this work, we present a new technique for intravenous injection and blood sampling suitable for rats and mice, which seems to be minimally traumatic while also easily performed.

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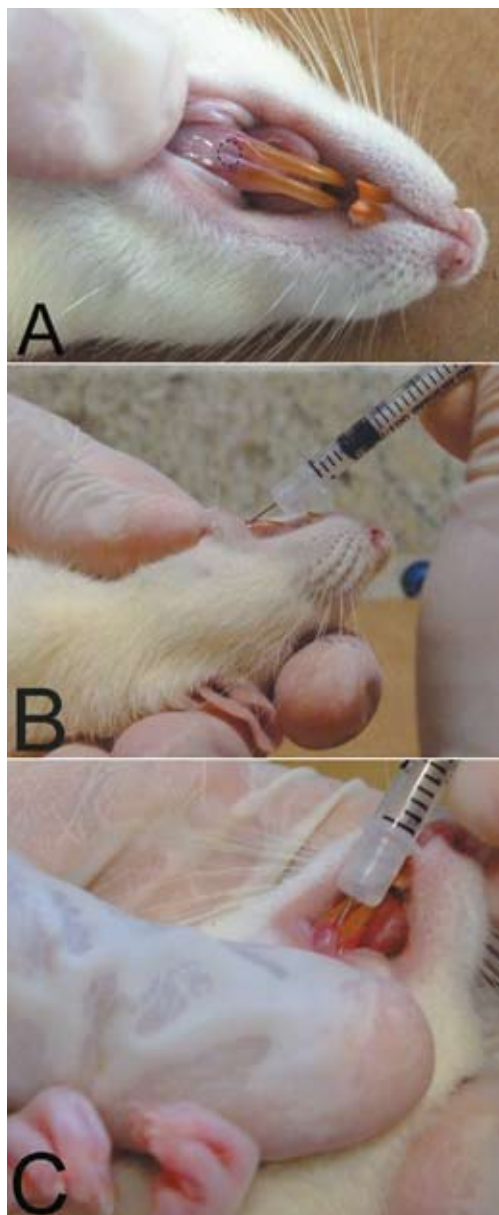
### *Technique Description*

A study demanding intravenous injection in female rats was the starting point for the development of this technique. In addition, since our laboratory deals with nociception research, we also needed a method that must not be another source of pain and physiological distress. Thus, we were reluctant to use methods such as retro-orbital and tail vein injections.

The new technique we are proposing uses the labialis mandibularis vein located in the gingival papillae region just below the pair of mandibular incisors. Although developed for female rats, it proved to be a good alternative to the penile or caudal vein injection, and it is also easy to do in mice.

Under light anaesthesia with halothane the animal is held in the hand of the experimenter and maintained in a supine position. The inferior lip is pulled back to expose the gingiva below the inferior incisors (Figure 1A). Taking an angle of 20 to 25° along the line between the pair of incisors, a 28-30 gauge needle is inserted about 2 mm into the gingiva (Figure 1B and C).

This technique allows approximately 800 µl of blood to be sampled over a 1-min period of a single sampling event. Also, large volumes (about 1000 µl as a bolus) can be administered. These data were



**Figure 1.** Site and details of the gingival vein puncture. **A-** The gingival area localized after the lower lip retraction. Dotted circle shows the injection site. **B and C-** The completion of the procedure. Note that only a small part (2 mm long) of the needle is inserted with an angle of 20-25° degrees.

obtained in tests with female/male rats weighing 300 – 350 g. We also tested this technique in mice (40 – 50 g) and approximately 100 µl of blood over a 1-min period could be sampled, while a volume of 150 µl as a bolus could be injected.

### Discussion

Table 1 shows the most frequently used vascular routes for blood collection or substance administration in laboratory animals. It is important to note that some features of these routes directly influence the outcome of the work, either the quality of the sampled/injected material or the physical/behavioural response of the animal used. Therefore this table shows some considerations that should be evaluated at the time of choosing the vascular route for a study.

Among some characteristics of the routes we highlight the potential for serial collections in the same animal, since this is an important feature for pharmacokinetic studies and clinical analyses. An optimal blood collection method will also depend on whether the sample must be taken aseptically (*Hoff, 2000*). We considered that an aseptic collection is one where blood has no contact with external means, or is collected directly to the appropriate container. Another point was the definition of the blood type offered by each route, since in most cases the purpose of the work requires exclusive use of the arterial or venous blood.

Whenever animals are used in laboratories, minimizing pain and distress should be as important an objective as achieving the experimental results (*Morton et al., 2001*). It involves not only the humanitarian concern aspect but also fidelity of the results, since animal harm exerts influence. We then categorized the level of impact on animal welfare after examining the techniques necessary to perform each route and compared with the proposals from guidelines and workshops for use of laboratory animals (*National Research Council Institute for Laboratory Animal Research, 1996; Diehl et al., 2001; Morton et al., 2001*) [4,5,7].

**Table 1.** General features of the commonly used routes.

<b>A) For removal of blood</b>					
<b>Route</b>	<b>No. of punctions</b>	<b>Aseptic blood collection</b>	<b>Blood type</b>	<b>Anaesthesia</b>	<b>Welfare Impact</b>
Saphenous vein	Repeated	No	Venous	No	••
Dorsal pedal vein	Repeated	No	Venous	No	••
Tail vein (punction)	Repeated	Yes	Venous	No	••
Tail vein (section)	Repeated	No	Mixture	No	•••
Retro-orbital	Repeated	Yes	Venous	Yes	•••
Jugular vein	Repeated	Yes	Venous	Yes	••
Cardiac puncture	Single	Yes	Venous, arterial or mixture	Yes	•••
Posterior vena cava	Single	Yes	Venous	Yes	•••
Axillary vessels	Single	No	Mixture	Yes	•••
<b>Gingival vein</b>	<b>Repeated</b>	<b>Yes</b>	<b>Venous</b>	<b>Yes</b>	<b>•</b>

<b>B) For substances administration</b>			
<b>Routes</b>	<b>Anaesthesia</b>	<b>Welfare Impact</b>	<b>Comments</b>
Penile vein	No	••	Limited to male animals. May cause edema, bleeding and discomfort at the site of injection.
Tail vein	No	••	Might need to warm the animal to dilate vein.
Jugular vein	Yes	••	Difficult to precisely locate the vein, might need fur removal.
Retro-orbital	Yes	•••	Ocular ulcerations puncture wounds, loss of vitreous humor, infection, keratitis or blindness may occur.
<b>Gingival vein</b>	<b>Yes</b>	<b>•</b>	Allows to multiple injections with limited gingival damage.

• Less impact: e.g. not painful, minimal invasive procedure, quick.

•• Medium impact: e.g. painful, restraint stressful or possible damage to surrounding tissues.

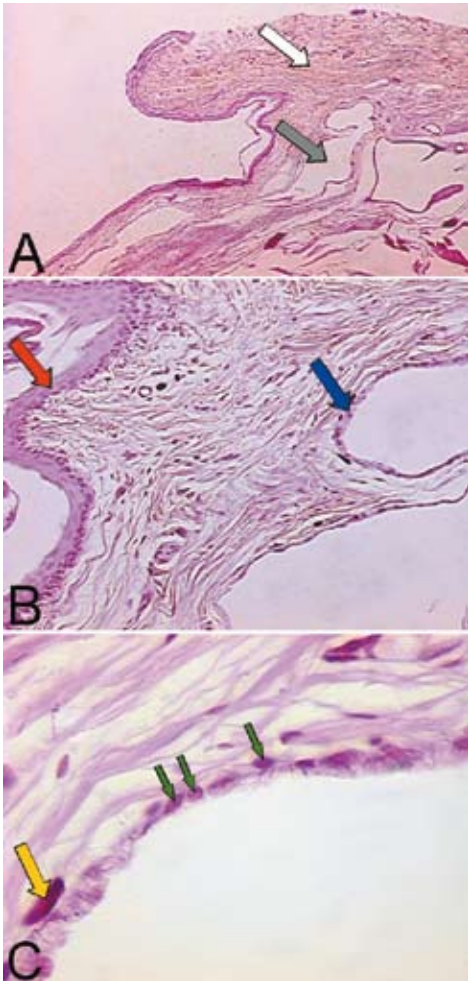
••• Maximum impact: e.g. painful, restraint stressful, serious injury or death.

Note: The scoring system refers to the physical and behavioural effects in animals caused by the use of each route, and assumes that the techniques are carried out by trained and competent staff with appropriate resources.

The gingival vein punction seems to agree the most with recommendations to maximize the quality and applicability of results while preserving animal well-being. Anatomic correlations and histological analysis (Figure 2) confirm that the vessel used in this technique is the labialis mandibularis vein.

Its venous drainage goes to the superior cava vein and the right heart, circulating in the lungs (small circulation) before its body distribution, which may be especially interesting for studies involving direct pharmacological access to the heart or lungs.

Owing to the large caliber of this vein, it is possible



**Figure 2.** Gingival photomicrography and its morphological characteristics. **A-** The white arrow shows the lamina propria while the gray arrow shows the labialis mandibularis vein (20x). **B-** Red arrow shows the stratified squamous epithelium. The blue arrow shows the labialis mandibularis vein endothelium (100x). **C-** A detail of the labialis mandibularis vein wall (400x). Yellow arrow shows the smooth muscle fiber nucleus, green arrows shows the endothelial cells nucleus. All images were obtained from the same slide. Specimen was stained by orcein. Images were taken with Olympus BX50 light microscope.

to perform either multiple drug administrations or blood collections in short periods of time without compromising study quality. Also, owing to the superficial location of the labialis mandibularis vein the method is minimally invasive causing little local tissue damage.

### Conclusion

In the present work a new technique for intravenous injection and blood sampling in rats was shown. This procedure is simple, precise and causes minimal harm to the animal. These characteristics suggest that there is minimal pain or stress after the recovery from anaesthesia. Therefore this procedure may be considered a good substitute for other traditional methods.

### Acknowledgment

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*National Centre for the Replacement, Refinement*

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