Collecting Blood from Rodents: A Discussion by the Laboratory Animal Refinement and Enrichment Forum

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"In your own experience, what is the least disturbing technique - from the subject's point of view! - of blood collection for rodents" (Reinhardt)?

"If we only need a small amount of blood and want to avoid disturbing our small rodent patients we usually draw a sample from the saphenous vein. I think, this is the least distressing approach for the animals" (Lang).

"It's been some time since I've done blood collection in rodents, but I found that for mice the least disturbing method was the retro-orbital bleed, sometimes with the help of a little anesthetic if the animals were of a feisty strain. As for rats, I think it really depends on how much blood is required for a sample. In my experience, tail clips were very effective when you needed just a view drops every now and then. This procedure took only a few seconds and the rats didn't seem to be in any apparent discomfort. But when one is looking for a good amount of blood, the jugular stick really did the trick. It provided quick results and required little time for 'hold-off', which the rats seemed to appreciate. They would go back into their boxes, groom a little and then get on with their routine" (Skoumbourdis).

"I used to take blood samples from the retro-orbital sinus in mice and got quite good at it. Fortunately no multiple bleeds were required on the same day. Now I am in a different department and need to take eight samples in 24 hours. It really bothers me to use this site at the eye so often. I believe the saphenous vein is the way to go, although it may take longer

in the beginning to become really proficient. I became aware of the saphenous procedure when Dr. Sally Walshaw from Prince Edward Island Canada came to speak at a meeting I was attending. There are important advantages to the saphenous draw:

1. No risk of cataract and blindness resulting from regular retro-orbital bleeding.
2. No anesthesia is required.
3. You can often use the same bleeding site just by dislodging the clot/scab.
4. Less stress for the rodent subject.
5. Esthetically more acceptable.
6. Sally suggested this as an ideal method to bleed not only mice but also hamsters, rats and guinea pigs. She took a series of photos demonstrating the saphenous blood collection procedure in mice" (Luzzi).

"A person working in ophthalmology told me once, that he didn't like the retro-orbital bleeding method at all because it can easily alter the intraorbital pressure causing severe discomfort to the subject. So yes, there are legitimate ethical concerns" (Weilenmann).

"To my knowledge retro-orbital bleeding is mainly used in mice, rats, hamsters and guinea pigs. This technique does have important advantages:

1. The technique is quick,
2. easy in skilled hands,
3. yields a relatively large sample (e.g., 1 ml from a rat).
4. The eyes can be used in alternation with one-week interval.
5. The rodent subject recovers quickly as reflected in corticosterone, catecholamine and behavioural responses (van Herck et al., 2001).

These practical advantages, however, are outweighed by serious ethical disadvantages:

1. The procedure is painful and, therefore, should never be done without proper anaesthesia.
2. There is a risk of complications, especially forward protrusion of the eyeball, caused by continuous bleeding from the retro-orbital venous plexus. This leads to a gradual drying out and a constant itching of the cornea, as eyelids are not able to close properly. The animal will react with excessive scratching, and by doing so will ruin its cornea. Within a short while you will find the animal with a blind eye.
3. The procedure is esthetically unpleasant" (Baumans).

"I heard my mouse-researcher friend mention one time that she stopped doing this collection technique. She said that she was never really good at retro-orbital bleeding and, therefore, felt very uncomfortable when she had to bleed a mouse. Fortunately, the issue became moot when a colleague introduced an alternative method. Drawing a sample from the maxillary vein is much easier and less risky for the mouse, but it often takes much more time to accomplish the job which in turn can stress the mouse quite a bit. For the retro-orbital bleeding, the rodent is usually anesthetized. This implies that your can get a blood sample very quickly IF you are proficient and skilled" (Kerwin).

"Although I will admit that they appear to be somewhat gruesome, if you have a good teacher and enough practice - this above all is the most important part of the puzzle - retro-orbital bleeds aren't a bad method. It's quick, provides a good amount of clean sample and, in my opinion, requires little to no anesthetic depending on how much your mice resist. Now, I will admit that errors can occur during these bleeds, and I myself have made a few that have ended up in a way that definitely did not sit with me well at all. But, at the time, it was the method I was told to use by my investigator so that's what I did. I was lucky enough to have a patient teacher who taught me to do it correctly, and I did my best to cause as little discomfort as possible to the little creatures" (Skoumbourdis).

"It's obviously not so much the actual procedure but the skill and sensitivity of the person who performs the procedure that is the key factor determining the safety plus well-being of the subject. As a clinical vet I would dare say that this is true for all hands-on procedures lab animals are subjected to. Even a simple routine injection can be experienced by the subject as an almost unnoticeable prick or as a very disturbing, perhaps even painful and distressing event depending on who is giving the injection" (Reinhardt).

"This is a very important point about clinical animal medicine and laboratory procedures in general. I believe that the most important part of learning in this field is finding a skilled, patient and caring teacher. I sincerely hope that everyone out there has found such an individual at some point in their career who has not only helped them to better understand the ins and outs of this field, but has shown them that there are many steps we can all take to make it a better place for the magnificent creatures who pay us such a great service - no matter what the procedure" (Skoumbourdis).

"The first time I saw a retro-orbital bleeding was about 5 years ago. We needed a sample to test for MHV. I called our vet and asked if he could teach us newbies how to get a blood sample from a mouse. He discussed various methods and then told us that he always did retro-orbital bleeding on mice. He then proceeded to do the deed, without any anaesthesia. It took maybe 4-5 seconds. I don't mind saying that I went completely weak in the knees and if I had not been standing next to a wall, I might even have gone down! We checked the mouse several times that day and he seemed fine, better than me in fact" (Conte).

"One of the problems with quick checks of prey animals in such circumstances is that it is possible they have evolved not to show signs of injury, especially in the presence of predators, which we humans are. This would make them more liable to attack. So, the mouse might have been suffering after the bleeding, perhaps considerably, but as soon as he saw a human face appear above the cage, he 'pretended' he wasn't in pain. The reason why animals have evolved a pain response is presumably to protect organs from further damage. Eyes are very important to mice, so I really struggle to think that mice would not have evolved a pain mechanism to protect and avoid further damage once the eyes had been injured. I'm sure if someone took blood retro-orbitally from me, it would hurt. I can only think that the same would be experienced by mice, even if we are unable to detect changes in their gross behaviour" (Sherwin).

"I am also sure that it must have been painful and I didn't realize that the mouse might have seen me as a predator. I don't allow retro-orbital bleeding in our animal facility anymore" (Conte).

"The main problem is that there can be serious damage to the eye and the bones of the orbit. Furthermore the blood loss afterwards cannot be stemmed, or quantified and while one does not see frank haemorrhage there must be some residual flow after the pipette has been withdrawn. The best route seems to be the jugular veins but it takes practice" (Morton).

"In Holland it is forbidden to take blood from the retro-orbital sinus without proper anaesthesia, as the procedure is really painful. One has to remember that the conjunctiva has to be penetrated during this procedure. Taking blood from the lateral saphenous vein or by a small cut in the ventral tail vessels can be done without anaesthesia and goes fast in mice and rats. I have little experience with gerbils but would assume they can be handled in the same way" (Baumans).

"I believe retro-orbital bleeding is no longer an acceptable procedure here in Canada. Side effects outweigh the benefits. As a general rule I would say that bleeds are done preferentially by saphenous vein in mice, by jugular vein in rats" (Moreau).

"I have used jugular veins as well. It's a good approach if a relatively large amount of blood is required. To draw blood from a jugular vein is easiest when the animal is under anesthesia, although I have taken samples with no problem on awake rats. In my experience gerbils and hamsters are one of the hardest to do a jugular stick on because of the amount of excess skin" (Lang).

"From what I understand, people are moving away from the retro-orbital towards the mandibular bleed as a standard for collection in mice and it is currently the standard used for transgenics. This might say to many that the retro-orbital could be nearly phased out. However, for those doing research involving the vascular system of the eye, I would feel that the retro-orbital bleed would still be the best method of collection" (Skoumbourdis).

"I am not that enthusiastic about mandibular bleeding. I tried this technique myself, and even under slight anaesthesia the mouse responded with a movement which suggested that it was feeling some pain. Furthermore, the location of the needle injection is a tricky area, as the parotic duct is running very close to the maxillary vein. I dissected a mouse and found quite an extensive haemorrhage around the vein and parotic duct on the mandibular muscles. Personally, I prefer the saphenous vein approach as it is less invasive" (Baumans).

"In summary, it's fair to conclude the clinical and ethical concerns outweigh the practical advantages of the retro-orbital bleeding technique in rodents. Preference has to be given to alternative techniques, especially to the saphenous blood collection procedure, that are less risky and less painful to the subject. Proper training of the animal technician by a skilled, patient and compassionate teacher is the key condition to protect the rodent subject from undue stress, discomfort and pain during the enforced restraint and the actual venipuncture, independent of the site from which the blood is then collected" (Reinhardt).

REFERENCES

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