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Venipuncture Site Influences Blood-drop Volume in C57BL/6 Mice

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Many experiments require the collection of serial blood samples from mice. However, the size of mice limits the volume of blood that can be safely collected as a survival procedure. In IACUC protocols, investigators may report the amount of blood they collect from mice as a number of drops. Many institutions, including ours, use an anecdotal conversion factor (1 drop of mouse blood = 25 µL) to ensure that blood-collection volumes are compliant with institutional guidelines. To our knowledge, previous work has not experimentally determined the volume of a drop of mouse blood. In this 10-wk crossover experiment, 2 phlebotomists bled 30 C57BL/6J mice from 3 sites (facial, saphenous, and tail) using one or 2 different needle gauge sizes per site. Male and female mice were weighed weekly and divided among 5 groups (n = 6): left and right tail vein, left and right saphenous vein, and facial vein. A single blood drop from each site was weighed, and the volume of each drop was calculated using the average blood density determined from 8 mice terminally bled at the end of the study. Venipuncture site and side significantly influenced blood-drop weight and thus calculated volume. Facial vein puncture produced the largest drop volume (mean: 21.7 µL), followed by the saphenous vein (mean: 9.97 µL) and tail vein (mean: 4.96 µL). Collection from the facial vein was associated with more hemorrhage and morbidity. Left-sided venipuncture was associated with slightly larger-volume blood drops, though the effect size of side was small. The results of this study may be useful in more accurately estimating blood loss via conversion of drops to volume. Our data indicate that blood collection from saphenous and tail veins minimizes blood loss relative to facial vein puncture and may optimize both serial collection of small-volume blood samples and animal welfare.

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Introduction

Mouse phlebotomy is complicated by their small size and limited peripheral venous access. Despite the small total blood volume of mice, serial blood samples are often needed to study drug pharmacokinetics or monitor changes in blood parameters over time.[26,27] However, excessive and/or chronic blood loss can result in anemia, hypovolemic shock, and death.[21] To reduce the risk of these complications, the NC3Rs and many institutional animal-use procedures recommend that no more than 10% of the total blood volume (average 72 mL/kg in mice) be collected from a single mouse at any one time.[21,22] The NC3Rs guidelines for serial blood collection in mice recommend that no more than 15% of total blood volume be collected within a 28-d interval and suggest a limit of less than 1% of total blood volume be collected in 24 h for serial samples obtained at short intervals.[21]

Investigators need to report in IACUC protocols the amount of blood to be collected and potentially lost at each time point and carefully monitor the volume of blood lost at each collection. Because blood samples from peripheral venous sites are frequently collected directly into a microcentrifuge tube or capillary tube (but rarely a volumetric syringe), the amount of blood collected is often reported as a number of drops. Many institutions, including ours, use the conversion factor that one drop of blood is equivalent to a volume of 25 µL to calculate the total volume of blood collected.[4,14] This conversion factor may have been extrapolated from a previous study which determined that the mean volume of a drop of saline solution was 25 µL when dispensed by a 30-gauge needle.[25] The diameter of a 30-gauge needle is comparable to that of a mouse tail vein, perhaps leading to the adoption of this conversion factor.[6,11] One study reported that the volume of a human blood drop was approximately 25 µL.[5] Another study subjectively defined a small drop of mouse blood as 15 to 20 µL.[16] However, the authors of the present study are unaware of previous reports of the measured volume of a drop of mouse blood. Furthermore, the conversion factor used by our institution and others may not be applicable across all common venipuncture sites.

The purpose of this study was to evaluate the accuracy of the drop to volume conversion factor used by our institution. In addition, we evaluated how venipuncture site, mouse sex, mouse weight, needle gauge, and phlebotomist impacted murine blood-drop volume. Results from this study can be referenced to determine guidelines for survival blood collection in C57BL/6J mice. Results from this study may guide investigators to favor one venipuncture site over another based on the blood volumes they require at different time points.

Materials and Methods

This study was performed under an IACUC-approved protocol at an AAALAC-accredited institution in compliance with the *Guide for the Care and Use of Laboratory Animals*.[9]

Animals and husbandry. C57BL/6J mice (15 males and 15 females) were bred in house from 4 breeding trios. Mice born to these trios but not initially enrolled in this study were maintained as alternates in case experimental mice required

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replacement. All mice were born within a 7-d period and were ear-punched before 14 d of age to identify individual mice. At the end of the study, any remaining mice were transferred to other experimental and training protocols.

Mice were housed in individually ventilated single-filter top cages (Allentown, Allentown, PA) filled with 1/4-in. corncob bedding (The Andersons Lab Bedding Products, Maumee, OH), a disposable hut (Bio-Serv, Flemington, NJ), and nesting material (Ancare, Bellmore, NY). Mice were group-housed by sex with 3 mice/cage, with the exception of one male that had no male littermates and could not safely be housed with other males. Mice were fed irradiated pelleted feed ad lib (Teklad Diet 7912; Inotiv, West Lafayette, IN) and received water bottles filled with tap water. Cages were changed biweekly. Room temperature was maintained between 71 and 73 °F (21.7 and 22.8 °C) with humidity between 30 and 70%. The room was maintained on a 14:10 h light:dark cycle with lights on at 0600 and off at 2000. Blood was collected from mice between 0800 and 1100. Mice were housed in an SPF facility as determined by triannual health surveillance that includes both environmental and sentinel samples. Mice in this facility routinely test positive for *Klebsiella pneumoniae* and *K. oxytoca*, mouse norovirus, *Rodentibacter heylii* and *R. pneumotropicus*, Beta hemolytic *Streptococcus* group B, and *Helicobacter* spp. The following pathogens are excluded from this facility based on historical health monitoring reports: *Streptococcus pneumoniae*, *Streptobacillus moniliformis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Mycoplasma pulmonis*, CAR bacillus, *Clostridium piliforme,* Beta hemolytic *Streptococcus* groups C and G, *Citrobacter rodentium*, *Bordetella hinzii* and *B. bronchiseptica*, *Campylobacter* spp., *Corynebacterium bovis* and *C. kutscheri*, *Pneumocystis carinii* and *P. murina*, Sendai virus, murine norovirus, mouse hepatitis virus, pneumonia virus of mice, minute virus of mice, mouse parvovirus, reovirus, mouse rotavirus, ectromelia virus, lymphocytic choriomeningitis virus, polyoma virus, K virus, mouse adenovirus type 1, Theiler murine encephalomyelitis virus, endoparasites, and ectoparasites

Study design. At 8 wk of age, mice were assigned to one of 5 groups (n = 6, 3 males and 3 females per group). Using a crossover study design, groups were randomly assigned to one of 5 treatments every week for 5 wk. The order in which mice in a group underwent the assigned treatment was also randomized. This study was repeated for a second 5-wk period (10 wk total) performed by a second phlebotomist. The first phlebotomist (#1) is a board-certified laboratory animal medicine veterinarian who works primarily with large, USDA-covered species. The second phlebotomist (#2) is a registered laboratory animal technician who regularly works with mice. Approximately 1 wk before the study initiation, both phlebotomists attended a 1-h training session during which they practiced the facial, saphenous, and tail venipuncture techniques using mice that were available for training purposes. The phlebotomists had no previous experience with saphenous venipuncture but were experienced with tail and facial venipuncture. As part of their employment responsibilities, phlebotomist #2 performs facial venipuncture more routinely than does phlebotomist #1.

The 5 experimental treatments included venipuncture via the 1) saphenous vein with a 25-gauge needle, 2) saphenous vein with a 27-gauge needle, 3) tail vein with a 20-gauge needle, 4) tail vein with a 23-gauge needle, and 5) facial vein with a 20-gauge needle. As described in a previous study, facial venipuncture in mice may target many vessels in the anatomic region of the caudolateral cheek.[23] For the purposes of this study, we refer to the vessel targeted during caudolateral cheek puncture as the facial vein. For the saphenous and tail-vein collections, groups were randomly assigned to be bled from the left or right side.

Previous work has shown that facial venipuncture induces significant fibrosis and subcutaneous hemorrhage at the venipuncture site.[1,8,20,28] To reduce confounding effects of these pathologic changes on the blood-drop volume after repeated facial venipuncture, each side of the face of each mouse was only bled once. Phlebotomist #1 is right-hand dominant and therefore restrained mice with their left hand and bled the right facial vein. Phlebotomist #2 is left-hand dominant and therefore restrained mice with their right hand and bled the left facial vein. Therefore, each side of the face was bled only once for each mouse.

Approximately 1 mo before data collection began, the analytical balance used in this study was serviced by a technician and its accuracy validated (Model XPE105; Mettler Toledo, Columbus, OH). Mice were weighed every week in a tared 50-mL conical tube that had a hole punctured at its end for airflow (Figure 1A). Weighing mice in this conical tube reduced movement artifact and took less than 60 s per mouse.

Blood collection. For mice undergoing saphenous or facial venipuncture, a weigh boat was weighed before blood collection. Mice undergoing saphenous venipuncture were restrained using a 50-mL conical tube with a hole for airflow at the end (Figure 1B). The assigned hindlimb was shaved, and the lateral saphenous vein was identified before puncturing the vessel with the assigned needle gauge. The first drop of blood that formed at the puncture site was dropped on to the preweighed boat, and the weight of the blood drop and weigh boat were immediately recorded. Mice undergoing facial venipuncture were manually restrained, the assigned facial vein punctured, and the first blood drop was collected and weighed as described for the saphenous venipuncture group.

For blood collection from the tail vein, a glass microhematocrit tube (Fisher Scientific, Houston, TX) was weighed before blood collection, and mice assigned to this treatment were placed in a restraint device, as shown in Figure 1C (Kent Scientific, Torrington, CT). A marker was used to mark the tail approximately 5 cm distal to its base. A hand warmer (HotHands, Dalton, GA) was applied to the exposed tail for 10 s, and then the cutting edge of a 20- or 23-gauge needle was used to make a small incision over the left or right lateral tail vein at the level of the mark. Blood was allowed to collect at this incision while the phlebotomist applied gentle manual pressure to the more proximal tail to "milk" the vessel. After 5 s, the preweighed microhematocrit tube was applied directly to the blood drop for 1 s, and the blood drop and microhematocrit tube were weighed immediately.

If a blood drop was not produced from the initial puncture, the phlebotomist would attempt collection from the site for a maximum of 5 attempts. The number of attempts was recorded. We also recorded the order in which mice from a particular group were bled. This information was used to evaluate associations between blood-drop volume and the number of venipuncture attempts or the order in which the mouse was bled. If any puncture site continued to bleed after drop collection, the phlebotomist applied pressure to the puncture site using gauze, and the mouse was returned to its cage after hemorrhage resolved.

Approximately 2 wk after the last day of data collection, 8 mice were randomly selected to undergo terminal cardiac blood collection. Mice were euthanized in accordance with the 2020 *AVMA Guidelines for the Euthanasia of Animals*. Mice were euthanized via carbon dioxide inhalation at a displacement

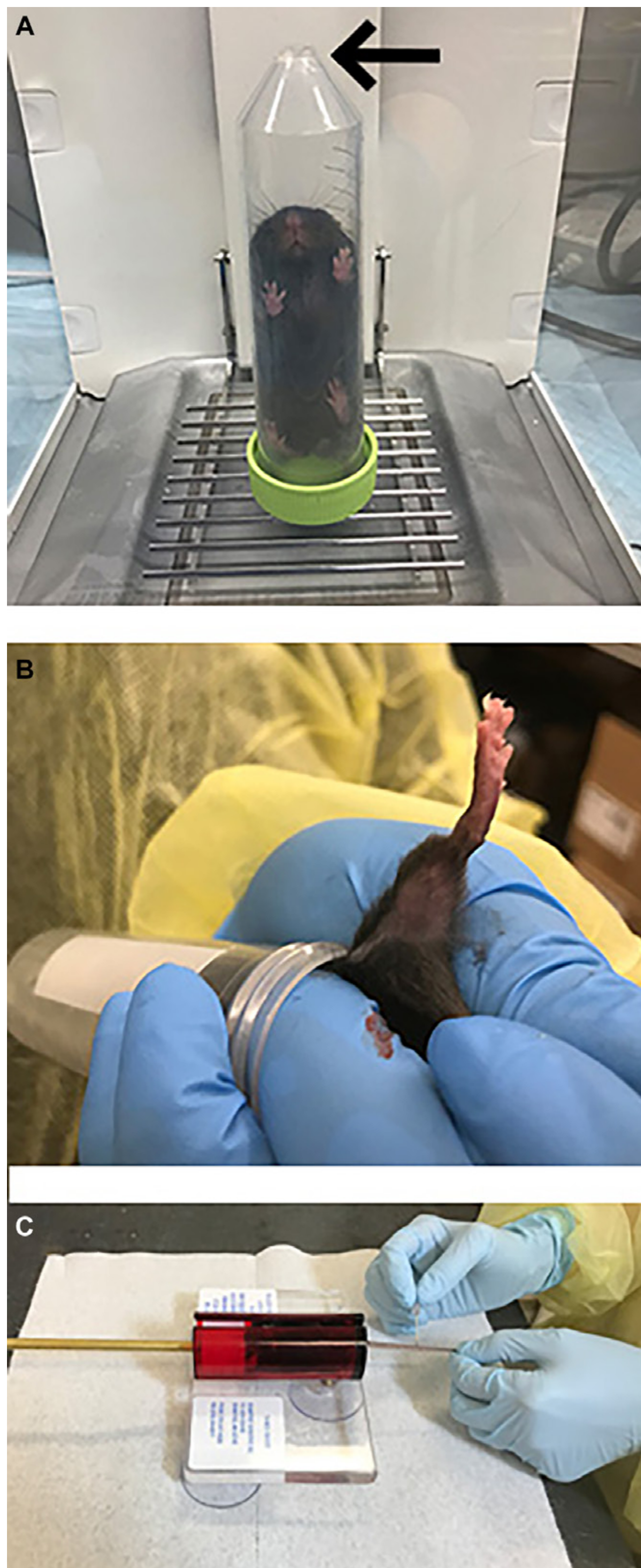

Figure 1. Mouse restraint for weight measurement and venipuncture. (A) Mice were weighed in a pretared conical tube with a hole at the end of the clear plastic for airflow (arrow). Weighing mice in this manner allowed for sufficient mouse restraint to reduce movement artifact that would impact accurate weight measurement. Mice were restrained for less than 60 s. (B) Mice were restrained in a conical tube with an airflow hole with a hindlimb exposed for saphenous venipuncture. (C) Mice were restrained in a device that facilitated tail access for tail-vein blood collection. In images B and C, the cranial aspect of the mouse is on the left side of the image and caudal on the right.

rate of 30 to 70% chamber volume/min. Immediately after cessation of respiratory movement, an insulin syringe (Cardinal Health, Dublin, OH) was used to collect up to 0.5-mL blood from the heart followed immediately by cervical dislocation. Immediately after cardiac collection, a micropipette (Eppendorf, Enfield, CT) was used to aspirate 100 µL of blood into a preweighed 200-µL pipette tip (Laboratory Products Sales, Rochester, NY), after which the tip and blood were weighed again. The weight of 100 µL of blood from each mouse was used to calculate a blood density for each sample. Given the similar blood density across the 8 samples, the densities were averaged and this value used as a conversion factor to determine the volume of the blood drops collected during the 10-wk experiment.

Statistical analysis. A sample size calculation was not performed for this study because this study is the first to our knowledge to assess murine blood-drop volume, and thus we could not anticipate the effect size. Instead, sample sizes were selected to be consistent with other publications that investigated blood collection in mice.[10,17]

Blood-drop volume was analyzed using linear mixed-effects models. The models included fixed effects of treatment (site and gauge), side of the body, an interaction between site and side, and study week, and a random effect of mouse due to the repeated blood draws taken on each mouse. Study week was correlated with mouse weight, which was recorded weekly and expected to increase over time as the mice aged. Additional covariates considered included sex, mouse weight, the number of attempts required to obtain the blood draw, and phlebotomist. Fixed effects were tested using F tests with a Kenward-Roger degree of freedom approximation and post hoc pairwise comparisons were performed using the Tukey HSD method. Cohen d effect sizes were reported for categorical predictor variables. Model assumptions of normality and homogeneous variance were assessed visually using residual plots. P values less than 0.05 were considered statistically significant. All analysis was performed using the R statistical software package.[2]

Results

Reliability of our institutional drop-volume estimate. A total of 294 drops of blood were obtained via the 5 phlebotomy treatment groups by the 2 phlebotomists. The mean drop volume across all treatments was 10.3 µL (Table 1). Drop volume ranged from 0.105 to 57.1 µL. The majority of blood drops obtained (95.2%) had volumes less than our institutional estimate of drop volume of 25 µL. All blood-drop volumes obtained from the tail vein (n = 120) and 113 of 114 drops obtained from the saphenous vein were less than our institutional estimate of 25 µL estimate (Table 1). Of the 60 blood drops obtained from the facial vein, 47 were less than 25 µL. Therefore, 100% of tail blood drops, 98% of saphenous blood drops, and 78% of facial blood drops did not exceed our institutional estimate of blood-drop volume. The average blood density calculated from the 8 mice that underwent terminal cardiac puncture was 1,048.99 ± 5.15 (SE) mg/mL.

Venipuncture site and drop volume. The interaction between site and side was not statistically significant (P = 0.748), and this interaction was therefore removed from all subsequent models. In the reduced model, the main effect of treatment (venipuncture site and needle gauge) was statistically significant (P < 0.001). Tukey comparisons between treatments indicate that there was a statistically significant difference between the blood-drop volume obtained from the facial,

Table 1. Mean and median drop volumes for each site

	Facial	Saphenous	Tail
Left side: drop volume (µL)			
Mean (SD)	22.10 (5.74)	10.60 (4.40)	5.47 (4.44)
Median [min, max]	21.60 [11.40, 33.40]	9.17 [1.91, 25.20]	4.38 [0.23, 21.40]
Missing[a]	0 (0%)	4 (6.70%)	0 (0%)
Right side: drop volume (µL)			
Mean (SD)	21.3 (11.60)	9.38 (4.49)	4.45 (3.60)
Median [min, max]	19.00 [6.79, 57.10]	8.83 [2.15, 22.30]	3.27 [0.11, 16.00]
Missing[a]	0 (0%)	2 (3.30%)	0 (0%)
Overall drop volume (µL)			
Mean (SD)	21.70 (9.11)	9.97 (4.46)	4.96 (4.06)
Median [min, max]	20.50 [6.79, 57.10]	8.88 [1.91, 25.20]	3.80 [0.11, 21.40]
Missing[a]	0 (0%)	6 (5.00%)	0 (0%)

The mean and median drop volumes obtained from left and right sides are provided. The bottom 3 rows of the table show the overall values from both sides.
[a]Number of mice from which blood could not be collected from the specified site. Variables that were not statistically associated with blood-draw volume (needle gauge, animal user, week, animal sex, animal weight, and number of blood-draw attempts) are not specifically detailed in the table.

saphenous, and tail-vein sites regardless of gauge, but there were no statistically significant differences between the gauges used within a site (i.e., 25 or 27 gauge for saphenous and 20 or 23 gauge for tail-vein venipuncture). In addition, custom linear contrasts were used to make pairwise comparisons between the 3 venipuncture sites, and a Bonferroni correction was applied to test the P values. All 3 sites were statistically significantly different ($P < 0.0001$, Figure 2).

Cohen d values, used to measure effect sizes, were large (ranging between 0.759 and 3.23) for comparisons between saphenous, tail, and facial venipuncture, supporting the finding that venipuncture site significantly affected blood volume obtained. Overall, the largest blood volume was obtained from the facial site with a mean drop volume of 21.7 µL (SD, 9.11), followed by the saphenous venipuncture with a mean drop volume of 9.97 µL (SD, 4.46), and tail venipuncture yielded the smallest mean drop volume of 4.96 µL (SD, 4.06) (Table 1 and Figure 2).

Venipuncture side and drop volume. Venipuncture side (left or right) was also significantly associated with blood-drop volume in the reduced model ($P = 0.015$), though the Cohen d value for effect size was small (0.34) (Figure 3). The mean difference in drop volumes between left and right sides was 0.8 µL for the facial vein, 1.22 µL for the saphenous vein, and 1.02 µL for

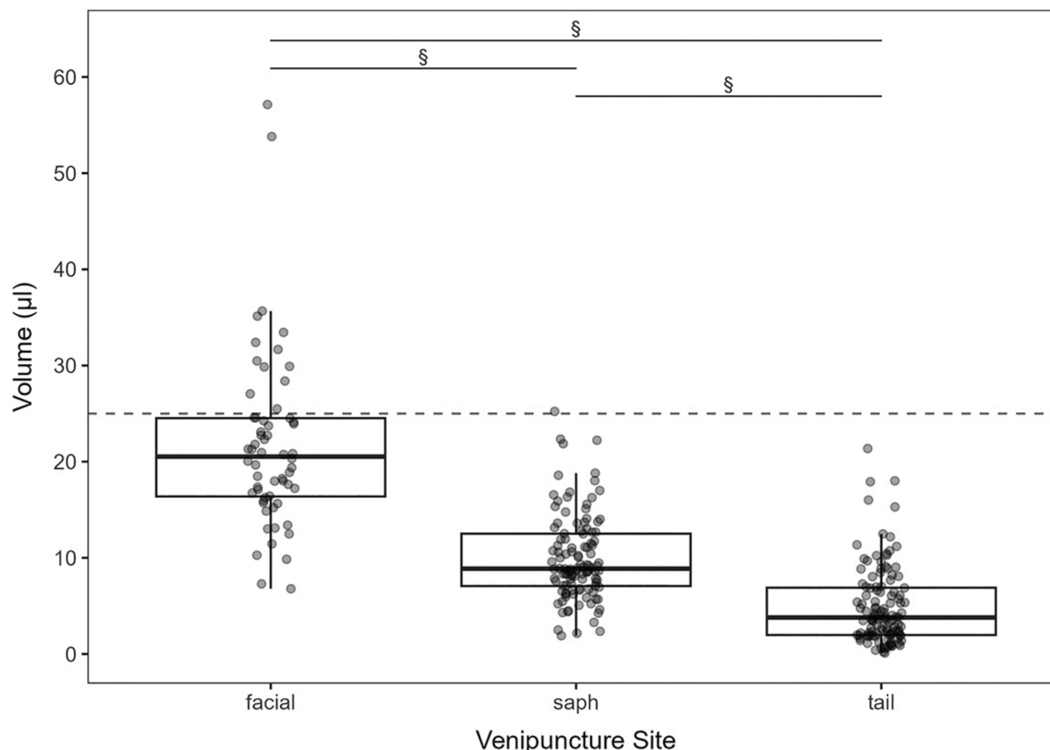

Figure 2. Drop volume by venipuncture site. Blood-drop volumes obtained from the 3 indicated venipuncture sites were significantly different (custom linear contrasts with Bonferroni correction; §$P \leq 0.0001$). The horizontal line indicates our institutional estimated blood-drop volume of 25 µL. Data shown are combined across nonsignificant variables (needle gauge, phlebotomist, week, animal sex, animal weight, and number of blood-draw attempts).

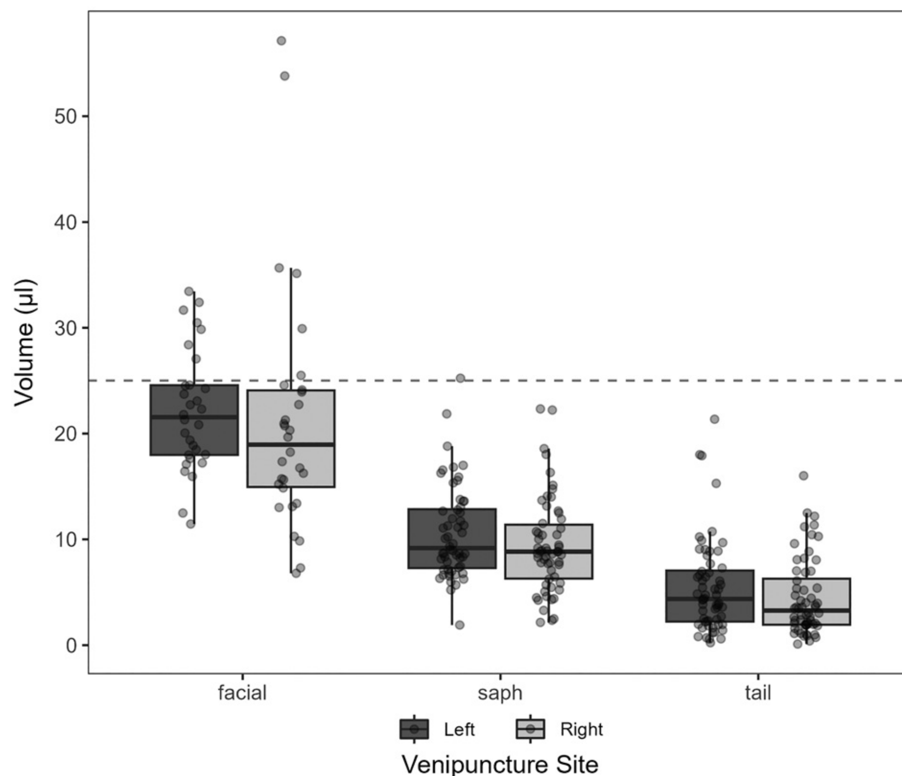

Figure 3. Drop volume by venipuncture site and animal side. There was a statistically significant association between blood volume obtained and venipuncture side (P = 0.015). The overall mean blood-drop volume was larger when obtained from the left side of the body by 1.82 µL. The horizontal line indicates our institutional estimated blood-drop volume of 25 µL. Data shown are combined across nonsignificant variables (needle gauge, phlebotomist, week, animal sex, animal weight, and number of blood-draw attempts).

the tail vein. Overall, left-sided venipuncture was significantly associated with larger blood-drop volumes, with a mean difference of 1.82 µL.

Impact of other variables on drop volume. Week was not a statistically significant effect (P = 0.090). There was no significant association between blood-drop volume and phlebotomist ($F_{1,8}$ = 1.7; P = 0.23), mouse sex ($F_{1,44}$ = 0.07; P = 0.79), mouse weight ($F_{1,50}$ = 0.14; P = 0.71), or number of venipuncture attempts ($F_{1,273}$ = 0.05; P = 0.82). Although phlebotomist was not significantly associated with blood-drop volume, phlebotomist #1, who was less experienced in mouse phlebotomy, had a greater range of blood-drop volumes obtained from the facial vein site (6.9 to 57.1 µL) when compared with phlebotomist #2 (11.4 to 33.4 µL) (Figure 4). The range of blood-drop volumes obtained between phlebotomists was similar for the saphenous and tail sites (Figure 4).

Feasibility and observed clinical impact of the phlebotomy from the 3 anatomic locations. On average, it took less than 2 attempts (mean, 1.53 attempts) to obtain a drop of blood. On average, 1.43, 1.76, and 1.35 attempts were needed to obtain a blood drop from the facial, saphenous, and tail sites, respectively. In 6 instances (2% of total attempts), blood could not be obtained. Four of these 6 instances were associated with an inability to obtain blood from the saphenous vein despite repeated attempts using a 25- or 27-gauge needle. In the remaining 2 instances, clippers used to shave the distal hindlimb before saphenous venipuncture inadvertently cut the skin, resulting in hemorrhage. In these 2 cases, venipuncture was not attempted, and the mouse was returned to its cage without blood collection.

During week 1, one mouse had a seizure after facial venipuncture. This mouse recovered (appeared alert, responsive, and ambulating) within 90 s after the seizure. During week 5, 2 different mice also had seizures after facial venipuncture. One of these mice recovered within 90 s after the seizure. The second mouse from week 5 was unresponsive and laterally recumbent with increased respiratory effort for approximately 3 min after the seizure; this mouse was euthanized to prevent further distress. This mouse was replaced in weeks 6 to 10 by an age-matched mouse that had not undergone any treatments in weeks 1 to 5.

Discussion

The results of this study suggest that the institutional estimate that one drop of mouse blood is equivalent to 25 µL will overestimate the amount of blood collected for the large majority of blood drops obtained from the facial, saphenous, or tail veins. However, some blood drops obtained from the facial vein may exceed the institutional estimate. Therefore, institutions and IACUCs may wish to increase their estimate of blood-drop volumes obtained from the facial vein to ensure compliance with NC3R blood-collection guidelines. In the authors' opinion, an overestimation may be advisable to account for the occasional loss of blood drops, continued hemorrhage after collection, and blood lost to internal tissue hemorrhage. Although a single-institutional conversion factor may be deemed sufficient to estimate total blood volume collected from a drop, blood-drop volume does differ significantly between the 3 anatomic sites assessed in this study. Although side was a statistically significant parameter, the difference in blood-drop volumes between sides is small regardless of venipuncture site and unlikely to be of clinical or practical significance.

Multiple studies have concluded that there is no superior blood-collection method or site in mice for all applications, and the pros and cons of each site should be weighed before selecting the site of venipuncture.[17,24] Furthermore, blood parameters

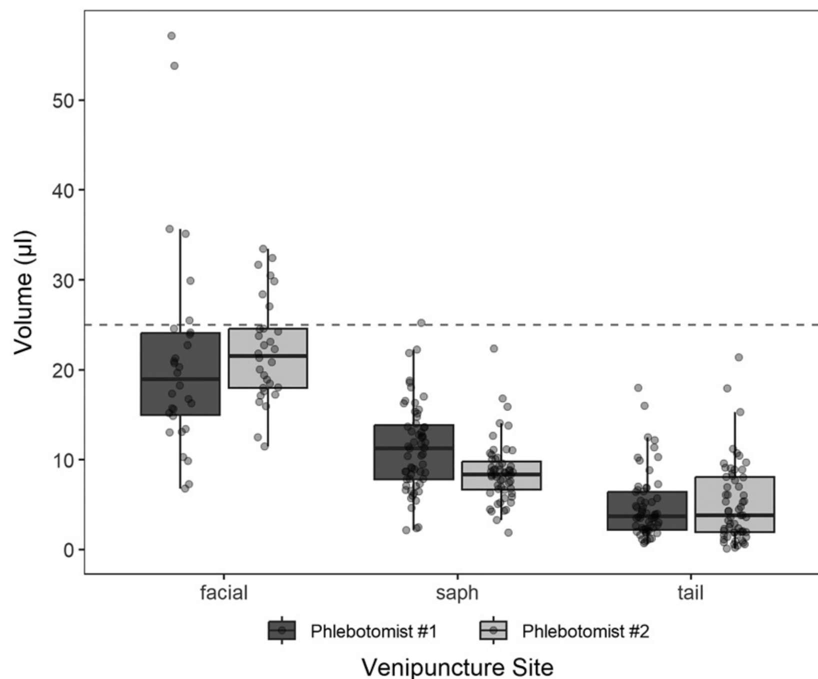

Figure 4. Drop volume by venipuncture site and phlebotomist. A linear mixed-effects model where phlebotomist was a fixed effect and week a random effect was used to test for statistically significant differences in blood volume obtained by the 2 phlebotomists. There was no association between phlebotomist and blood volume obtained ($P = 0.109$). Phlebotomist #1, who does not routinely bleed mice, had a greater range of blood volumes obtained at the facial and saphenous sites compared with phlebotomist #2, who routinely bleeds mice via the facial vein. This may suggest that prior venipuncture experience may influence the precision of blood volumes obtained. Our institution considers one drop of blood equivalent to 25 µL, which is indicated by the dotted line.

can differ among sampling sites and methods used for blood collection.[3,13] For example, one study found higher glucose values from tail-vein samples as compared with saphenous vein samples; this result may have been associated with the stress of prolonged restraint required to obtain large-volume tail-vein blood samples.[3]

In the present study, we observed that the facial vein puncture produced drops rapidly. Previous work has found that using the facial vein requires less time to collect blood than other venipuncture sites.[15] Although the facial vein may be advantageous when one needs to rapidly obtain large volumes of blood, this method likely increases the risk of extraneous hemorrhage and hypovolemia. In our study, the rapid flow of blood drops from facial vein puncture also made it more challenging to obtain only the first drop of blood. We suspect that the 2 largest volumes obtained during the study (57.1 and 53.8 µL), both from facial vein puncture, were calculated from 2 blood drops that fell indistinguishably on the weigh boat in rapid succession. Exclusion of these 2 largest drops from the data set did not impact the statistical analyses.

In addition, use of facial vein phlebotomy has been associated with severe histologic changes to tissues of the head and cheek, as well as evidence of increased pain, anxiety, and distress.[8,19,20] Repeated facial venipuncture has been associated with more serious clinical adverse events, such as mortality, convulsions, head tilt, and hemorrhage from the ear canal and nares.[8] Consistent with these prior reports, 3 mice in our study experienced generalized seizures immediately following facial venipuncture, and one of these mice required euthanasia. These convulsions have previously been attributed to the stress and/or trauma associated with facial bleeding.[8] However, other work has described a severe bradycardia and arrhythmias with the standard "scruff" restraint technique used to immobilize mice for facial venipuncture.[18] We did not evaluate cardiac parameters in this study, and future work should investigate if restraint technique for facial venipuncture contributes to adverse clinical outcomes.

Advantages of selecting the saphenous vein for blood collection include a lower risk for extraneous blood loss and exceeding total volume estimates and lower morbidity relative to facial vein puncture. In addition, the restraint device serves as a protective barrier between the mouse and phlebotomist. However, both reported ergonomic difficulties and hand strain using this restraint method, and the use of restraining devices may increase the overall procedure time and contribute to animal stress.[3] There also appears to be a trade-off between frequency of successful phlebotomy attempts and extraneous postcollection hemorrhage using different needle gauges at the saphenous site. In our study, all the missing data points are from mice assigned to saphenous venipuncture. Using a larger gauge needle may reduce the number of venipuncture attempts that do not yield blood but may also increase overall blood loss. Although the volume of drops obtained puncturing the saphenous vein with 25- and 27-gauge needles did not differ significantly, we anecdotally saw continued hemorrhage when the 25-gauge needle was used that required us to apply manual pressure to the vessel in most instances. In contrast, we saw little continued hemorrhage when using the 27-gauge needle, and manual pressure was rarely required to stop hemorrhage. The author has since trained to perform saphenous venipuncture using 23-gauge needles, which yields consistent blood collections, though does result in some extraneous blood loss due to hemorrhage postcollection.

The tail-vein phlebotomy site can allow for collection of the smallest blood volumes and, if a restraint device is used, also provide some physical protection for the phlebotomist. Some sources report that up to 300 µL of blood can be obtained from the tail vein, presumably by "milking" the punctured vessel until

the required blood volume is collected.[9] Collecting such large volumes of blood from murine tail veins can be time consuming, which may interfere with hematologic analyses. For example, slow blood collection can precipitate platelet clumping and sample clotting.[7] Furthermore, prewarming of the tail before bleeding, as performed in the present study, is associated with significant alterations in blood values, including WBCs and neutrophil and lymphocyte counts.[13] Despite these drawbacks, we did not note any adverse clinical events in mice after tail venipuncture. A previous study found that blood collection from the tail vein induced few alterations in physiologic and behavioral patterns in C57BL6J males, whereas facial venipuncture was associated with increased anxiety-related behavior.[19] These results support our finding that tail venipuncture is associated with improved welfare outcomes as compared with facial venipuncture.

We cannot predict how well results of the present study will extrapolate to other mouse strains and ages. For example, a previous study concluded that facial venipuncture was the "most humane method" of blood collection (as compared with tail and sublingual puncture) in 6-wk-old C57BL/6NTac mice.[12] Blood-collection techniques vary even within the same peripheral collection site (e.g., including a warming method for vasodilation); thus, drop volumes obtained from any given site might differ based on methodologic details. Furthermore, we included only 2 phlebotomists in this study. Comparisons among more phlebotomists, particularly those with different levels of experience, extend our findings regarding the effects of phlebotomist experience on drop volume. Future work should also investigate the volume of blood drops obtained from other peripheral sites, such as the submental site, and via alternative methods, such as tail-tip amputation.[10,20,23]

In conclusion, this study found that blood-drop volume in C57BL/6 mice is significantly different depending on the site of blood collection. Results from this study suggest that although no blood-collection site or method is superior, the saphenous and tail-vein sites may be preferred to minimize hypovolemia during serial blood collection, and the facial site should be avoided when possible to prevent extraneous hemorrhage, secondary hypovolemia, and adverse clinical outcomes. Clinicians and investigators should use discretion when choosing a venipuncture site in mice that optimizes both clinical and research outcomes. In addition, regulatory bodies, such as IACUCs, should consider how venipuncture site influences the guidelines they set forth for murine blood collection.

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Conflict of Interest

The authors have no conflict(s) of interest to declare.

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