Refining Blood Collection Techniques to Improve Animal Welfare and Sample Quality

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Abstract

An effort to refine rodent blood collection techniques resulted in an examination of alternative methodologies to improve animal welfare and sample quality. Disease model or repeated sample collection resulted in inadequate sample volume, occasional hemolysis, and complications including bruising, scarring, and necrosis of sample collection sites. A study was designed to determine whether the use of a vibrating device in comparison to standard straight stick methods would decrease health complications as well as improve sample quality and quantity. C57BL/6 mice were used as a control strain for both tail and saphenous venipuncture. NOD mice were used for the diseased tail stick groups. Atherosclerosis induced ApoB mice were used as a disease model for saphenous venipuncture. Tail sticks were performed twice daily for two months and saphenous bleeds were performed bi-weekly for two months, to mimic study schedules. Sample volume increased with the use of the vibrating device and blood collection was achieved without the use of heat, unlike the standard direct venipuncture method. Comparison of the two bleeding methods in the ApoB strain showed that we were able to collect complete volume in 73% of samples via the standard method versus 84% with the use of the vibrating needle. Full sample volumes were collected with single sticks in 38% of attempts of the standard method versus 70% of attempts with vibrating needle in ApoB mice. An incidence of bruising was noted at 36% with standard method versus 12% with the use of vibrating needle. Tail circumference at the site of standard venipuncture of C57Bl/6 mice averaged 13.2mm; this was a 3mm increase from both the control and vibrating needle groups which averaged 10mm tail circumference. This work demonstrates that, as compared to standard blood collection methods, the vibrating needle allows for increased sampling compliance while decreasing associated trauma.
Background

As an alternative to retro orbital eye bleeds, saphenous venipuncture is used with increased frequency in an attempt to decrease ocular complications, including blindness, corneal and retrobulbar trauma or loss of the eye. Saphenous venipuncture does not require the use of anesthesia, which could cause complications related to disease model or strain as well as alter blood chemistry. Complications from saphenous venipuncture can result in inconsistent blood sample volumes and localized post-bleed trauma - factors related to strain, disease model, and size of the animal.

Daily tail stick sample collection for blood glucose monitoring also causes multiple risks. SID and BID venipuncture of a non-obese diabetic (NOD) mouse tail increases incidence of bruising, swelling, and necrosis and may adversely affect animal welfare. Required monitoring can also disrupt the strain’s disease progression, as NOD mice can reverse to non-diabetic if infection is introduced.

Promising phlebotomy alternatives include the use of the GentleSharp device. The GentleSharp attaches to a needle hub and uses an oscillatory motion that reportedly requires less effort to pass through tissue. The reduction in force required to move the needle through tissue is believed to cause less trauma, stress and pain to the animals while allowing for adequate blood sample volume. A study was designed to compare standard versus GentleSharp saphenous and tail venipuncture blood collection methods in both normal and diseased mice.
<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Location of Collection</th>
<th>Collection Technique</th>
<th>Animals/Group</th>
<th>In-Life Period</th>
<th>Histology</th>
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<tbody>
<tr>
<td>1</td>
<td>C57 (female)</td>
<td>None</td>
<td>None</td>
<td>10</td>
<td>8 weeks in-life</td>
<td>Leg and Tail</td>
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<tr>
<td>2</td>
<td>C57 (female)</td>
<td>Saphenous and Tail</td>
<td>Standard</td>
<td>10</td>
<td>5 mice – 4 weeks in-life, 5 mice – 8 weeks in-life</td>
<td>Leg and Tail</td>
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<tr>
<td>3</td>
<td>C57 (female)</td>
<td>Saphenous and Tail</td>
<td>GentleSharp</td>
<td>10</td>
<td>5 mice – 4 weeks in-life, 5 mice – 8 weeks in-life</td>
<td>Leg and Tail</td>
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<tr>
<td>4</td>
<td>NOD (female)</td>
<td>Tail</td>
<td>Standard</td>
<td>5</td>
<td>8 weeks in-life</td>
<td>Tail</td>
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<tr>
<td>5</td>
<td>NOD (female)</td>
<td>Tail</td>
<td>GentleSharp</td>
<td>5</td>
<td>8 weeks in-life</td>
<td>Tail</td>
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<td>Saphenous</td>
<td>Standard</td>
<td>32</td>
<td>2 weeks in-life</td>
<td>NA</td>
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<tr>
<td>7</td>
<td>ApoB (male)</td>
<td>Saphenous</td>
<td>GentleSharp</td>
<td>23</td>
<td>2 weeks in-life</td>
<td>NA</td>
</tr>
</tbody>
</table>

NOD and C57BL/6 mice were allocated into Groups 2-5 (tail venipuncture). 27 gauge needles were used and changed between each animal. Venipuncture sites were indicated on the animals tail with permanent marker. Samples collected BID to mimic current diabetic study protocol.

ApoB and C57BL/6 mice were allocated into Groups 2, 3, 6, and 7 (saphenous venipuncture). 25 gauge needles were used and changed between each animal. Samples collected bi-weekly from the left saphenous vein to mimic atherosclerosis study protocol. Upon completion of the study, histology samples were collected from animals in groups 1-5.
ApoB mice are used to mimic human cardiovascular diseases. Throughout disease progression it becomes increasingly difficult to obtain full sample volume through saphenous venipuncture due to complications from altered metabolism, including atherosclerosis. Repeated venipuncture attempts often result in insufficient sample size and quality, bruising and scarring making future collections increasingly difficult.

The graph above indicates use of the GentleSharp over a two week period in ApoB mice improved complete sample collections in a single attempt as well as decreasing the amount of post-bleed bruising.
Saphenous Collection in Diseased ApoB Mice

Day 1 Bleed

- 28% of mice that had standard collection required the use of heat lamp
- Average sample collection size for standard collection was 181ul
- Average sample collection size for GentleSharp was 198ul

Day 14 Bleed

- 42% of mice that had standard collection required the use of heat lamp
- Average sample collection size for standard collection was 168ul
- Average sample collection size for GentleSharp was 191ul
Saphenous Collection in C57BL/6 Mice

A. Bruising noted one day post saphenous bleed with the use of the standard method. Animals in the GentleSharp groups had no evidence of bruising.

- Heat lamp not used for non-diseased mice
- Complete sample volume obtained for all collection method time points
Tail measurements were taken Day 1 and Day 56 using a Medline Surgical Ruler.
Figure A. indicates the use of the GentleSharp in non-diseased C57BL/6 mice increases tail circumference by 6% compared to the use of the standard method which increased tail circumference by 38%.
Figure B. indicates the use of the GentleSharp in NOD mice increases tail circumference by 17% compared to the use of the standard method which increased tail circumference by 47%
Comparison of Tail Venipuncture Methods in NOD Mice

A. NOD mouse post GentleSharp venipuncture.
B. NOD mouse post standard method venipuncture.

Along with increased tail circumference, the standard method increased the incidence of bruising in NOD mice.
No differences were observed between the standard collection and GentleSharp groups or between NOD and C57BL/6 mice. On day 56, microscopic lesions were characterized by focally extensive acanthosis (epidermal thickening), minimal to mild dermal inflammation and hemorrhage, and fibrosis in the dermis and subcutis, with extension into muscle, tendons, and nerves. (H&E stain, 100x magnification)
No differences were observed between the standard collection and gentle sharp groups in C57BL/6 mice. Microscopic lesions were not observed around the saphenous venipuncture site of either group on day 35. On day 56, lesions in both groups consisted of minimal to mild perivascular hemorrhage and neutrophilic inflammation. (H&E stain, 100x magnification)
Conclusions

- Study protocols were effectively mimicked by using strains specific to disease models.
- The use of the GentleSharp device for BID tail sticks effectively maintained tail circumference in C57Bl/6 mice while the use of the standard method increased circumference by 38%.
- The use of the GentleSharp device for BID tail sticks in NOD mice increased tail circumference by 17% while the use of the standard method increased circumference by 47%.
- The use of the GentleSharp device for bi-weekly saphenous venipuncture yielded a 72% success rate of single stick attempts versus a 40% success rate of single stick attempts while using the standard method in ApoB mice.
- Histological data indicates there was no significant difference in tail and saphenous vein puncture site trauma between the GentleSharp groups compared to the standard method groups.

Future Directions

- Determine the efficacy of the GentleSharp device for mouse mandibular blood collection
- Evaluate tissue pathology with the use of the GentleSharp on animals on long term diabetic studies to determine if it can decrease incidence of bruising in symptomatic NODs.

Acknowledgements

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