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Modified Blood Collection from Tail Vein of Non-Anesthetized Mice with Vacuum Blood Collection System and Eyeglasses Magnifier

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Dear Editor:

We would like to submit the enclosed manuscript entitled " **Modified Blood Collection from Tail Vein of Non-Anesthetized Mice with Vacuum Blood Collection System and Eyeglasses Magnifier** " for publication consideration in **JoVE**. The text includes 2 tables and 3 figures prepared using Microsoft Word Processing 2016 according to the journal's Instructions to Authors. We have provided all required supporting documentation.

Despite various methods already established for blood collection in mice, it is still not easy to obtain blood from non-aneasthetized mice. The authors had successfully collected the tail vein blood from non-anaesthetic mice by using the vacuum blood collection system, which reduced the risk of direct exposure to blood and made it easier to take multiple samples within a month. To our knowledge, this is the first report describing this method for collecting blood in mice. We hope this report would be interested by readers from JoVE.

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Yours sincerely,

Wusong Zou, M.D.

TITLE:

Modified Blood Collection from Tail Veins of Non-anesthetized Mice with a Vacuum Blood Collection System and Eyeglass Magnifier

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KEYWORDS:

mice, tail vein, non-anesthetized, blood sample collection, vacuum blood collection system, eyeglass magnifier

SUMMARY:

This study reports blood sampling from tail vein in mice using a vacuum extraction tube system with eyeglass magnifier. Our method is easy to practice and could be used for repeat blood sampling in mice.

ABSTRACT:

Blood sample collection is the basis of experimental animal research. It is of importance to obtain adequate blood samples for various research purposes. The tail veins of mice are small, and it is sometimes difficult to obtain the required blood volume by conventional puncture methods. This study investigates the superiority of repeated blood sample collection from tail veins of mice through use of a vacuum blood collection system and eyeglass magnifier (experimental group) compared to conventional blood sampling methods (conventional group), performed by beginners and experts, respectively. With the help of an eyeglass magnifier, a butterfly needle tip is inserted into the tail vein of each mouse in the experimental group. When the vein is

penetrated successfully, a blood sample is collected in the vacuum collection tube by insertion of the rubber end of a butterfly needle into the vacuum blood collection tube. The plunger is then used to collect blood without the help of the eyeglass magnifier in the conventional group. Success rates of blood sample collection by the beginners and experts were shown to be 70% vs. 100% ($p < 0.01$) in the experimental group and 35% vs. 85% ($p < 0.01$) in the conventional group. For both beginners and experts, puncture times required for obtaining required blood sample were significantly lower in the experimental group compared to the conventional group (2.40 ± 0.75 vs. 2.90 ± 0.31, $p < 0.05$; 1.15 ± 0.37 vs. 1.55 ± 0.76, $p < 0.05$). In conclusion, the presented blood sampling technique is feasible and easy to practice and enables frequent sampling of adequate blood volumes from non-anesthetized mice.

INTRODUCTION:

Blood sampling from animals involved in experiments is a basic research technique. There are some available techniques for blood collection from mice, including tail snips and puncturing of the heart, retro-orbital plexus, jugular vein, caudal vein, and vena cava. Ideally, blood should be collected in a minimally invasive manner, with minimal impact on the animal's health. However, the most commonly used techniques often inflict stress upon animals and can impact research outcomes[1]. Blood collection from the retro-orbital plexus can be used to obtain enough blood volume from mice[2]. However, it can result in severe tissue damage and does not allow for obtaining blood repeatedly in short time intervals[3].

The caudal vein is a superior location for blood collection, which inflicts minimal injury upon mice. However, the tail veins of mice are thin, and it is sometimes difficult to obtain enough blood through the conventional puncture technique. In some cases, repeated punctures are required to obtain the desired blood volume. Anesthesia is also commonly recommended to facilitate blood sampling from the tail veins of mice. Moreover, a scalpel, straight edge razor, or sharp scissors may be needed to remove the ends of the tails to obtain the required blood samples[4]. We have previously reported successful blood collection from the tail veins of non-anaesthetized rats by the vacuum blood sample collection system, which reduced the risk of blood contamination and avoided the need for repeated punctures[5]. This study reports a similar blood collection method in non-anesthetized mice.

PROTOCOL:

This study was approved by the Animal Care Committee of Wuhan Fourth Hospital (Wuhan, China) and maintained in accordance with the guidelines of ARRIVE (Animal Research: Reporting of In Vivo Experiments)[6] and the Guide for the Care and Use of Laboratory Animals of US National Institute of Health (NIH Publication No.85-23, revised 1996).

1. Husbandry

1.1. Use 12 week old Kunming mice.

NOTE: We used mice (n = 40, 20 males, 37–46 g, mean 42.38 ± 2.39 g) from the Experimental Animals Center of Tongji Medical College.

1.2. House the mice under standard conditions with free access to food and drinking water. Keep two mice in a 530 cm^2 cage with wood shaving bedding.

1.3. Maintain a room temperature between 21–23 °C.

1.4. Feed mice with a normal salt diet (0.3% NaCl) throughout the study.

2. Blood sample collection

2.1. Prepare the following equipment: vacuum tube (1 mL), butterfly needle, eyeglass magnifier, and plastic restraining holder. Place them on a sterile surface (**Figure 1**).

2.2. Place a mouse in a plastic restraining holder and wash its tail with warm water (20–30 °C). Wipe the tail with 70% ethanol-saturated cotton balls to expand the vein. Select the right or left tail vein for blood sampling. Grasp the lower portion of the tail gently and keep the tail straight during blood sample collection.

2.3. Collect the blood. If comparing methods, collect blood in two groups: the “experimental” group using the procedure we have developed, presented below, and “conventional” group using a conventional method, also presented below.

2.3.1. Experimental collection:

2.3.1.1. Wear an eyeglass magnifier to improve the viewing for puncture of the tail vein. Insert the 22 G butterfly needle tip into one of the lateral tail veins at a position approximately half the distance distally from the tip of the tail at an angel approximately 10°, moving towards the base of the tail for multiple samples.

2.3.1.2. Insert the rubber end of the butterfly needle into the vacuum blood collection tube to collect blood (**Figure 2**).

NOTE: If blood stops flowing out during blood collection, the needle angle should be quickly adjusted. To avoid blood coagulation in the needle, another puncture position should be selected if blood stops flowing out after 15 s.

2.3.2. Conventional method:

2.3.2.1. Insert the needle connected to a syringe into one of the lateral veins approximately one-third of the distance distally from the tip of the tail.

2.3.2.2. When blood appears in the hub, pull back the plunger slowly to collect blood (Figure 3)[7].

NOTE: To further elucidate the effects of varying experience with blood collection, a beginner and expert were chosen to collect blood samples using experimental and conventional methods simultaneously.

2.4. After blood collection, remove the needle and press the puncture point to stop bleeding. Then, release the mouse from the plastic restraining holder and return the mouse to its cage.

NOTE: It has been reported that up to 10% of total blood volume can be safely removed from a healthy animal at 2 week intervals[8], so about 175 µL of blood was collected each time in accordance with ethical principles.

2.5. Use tubes with EDTA to collect plasma and use tubes without anticoagulants to collect serum. Gently invert the tube several times and put them on ice vertically.

2.6. Centrifuge the blood sample collection tubes in a refrigerated centrifuge at 1,000 x g for 10 min to separate plasma and serum.

NOTE: Successful blood collection is defined as obtaining a volume of 175 µL each time. No more than three punctures should be attempted, and a failed blood collection is defined as a total blood volume of less than 175 µL after the third puncture. The sampling duration is defined as the time from the tail vein puncture to removal of the needle after blood collection.

2.7. Collect blood twice at intervals of 2 weeks[8].

3. Statistical analysis

3.1. Use commercially available statistical software for analysis. Present data as mean value ± standard deviation, using $p < 0.05$ as the cutoff for statistical significance.

REPRESENTATIVE RESULTS:

Body mass, blood collection volumes, and sampling durations of the two groups

Blood samples were collected from 20 mice (10 males) twice at 2 week intervals in each group. The mean body mass of mice was similar between the experimental and conventional groups for beginners and experts, respectively (42.40 g ± 1.42 g vs. 42.65 g ± 1.14 g, $p > 0.05$; 42.55 g ± 2.91 g vs. 43.20 g ± 2.69 g, $p > 0.05$). Collected blood volumes and sampling durations were similar between the two groups in experts (184.25 µL ± 11.95 µL vs. 171.75 µL ± 25.61 µL, $p > 0.05$; 1.85 min ± 0.68 min vs. 2.17 min ± 0.80 min, $p > 0.05$). However, higher collected blood volumes and shorter sampling durations were seen in the experimental group compared to the conventional group in beginners (172.00 µL ± 15.17 µL vs. 148.50 µL ± 30.22 µL, $p < 0.01$; 3.11 min ± 0.44 min vs. 4.08 min ± 0.61 min, $p < 0.01$). Compared to beginners, experts collected higher blood volumes and showed lower sampling times using experimental and conventional methods (184.25 µL ± 11.95 µL vs. 172.00 µL ± 15.17 µL, $p < 0.01$; 171.75 µL ± 25.61 µL vs. 148.50 µL ± 30.22 µL, $p < 0.05$; 1.85 min ± 0.68 min vs. 3.11 min ± 0.44 min, $p < 0.01$; 2.17 min ± 0.80 min vs. 4.08 min ±

0.61 min, $p < 0.05$) (**Table 1**).

Success rates and puncture times of the two groups

The comparison of success rates between beginners and experts was 70% (14/20) vs. 100% (20/20) ($p < 0.01$) in the experimental group and 35% (7/20) vs. 85% (17/20) ($p < 0.01$) in the conventional group. Higher success rates were also seen in the experimental group compared to the conventional group in beginners [70% (14/20) vs. 35% (7/20), $p < 0.05$]. In both beginners and experts, the number of punctures was significantly lower in the experimental group compared to the conventional group (2.40 ± 0.75 vs. 2.90 ± 0.31, $p < 0.05$; 1.15 ± 0.37 vs. 1.55 ± 0.76, $p < 0.05$). Compared to beginners, lower puncture times for experts were observed using experimental and conventional methods. (1.15 ± 0.37 vs. 2.40 ± 0.75, $p < 0.01$; 1.55 ± 0.76 vs. 2.90 ± 0.31, $p < 0.01$) (**Table 1**).

FIGURE AND TABLE LEGENDS:

Figure 1: Equipment. Shown are 1 mL vacuum blood collection tubes and a 22 G butterfly needle (left), an eyeglass magnifier (middle), and a plastic restraining holder (right).

Figure 2: Successful blood collection in the experimental group.

Figure 3: Successful blood collection in the conventional group.

Table 1: Comparison of results between the experimental and conventional groups. *$p < 0.05$, **$p < 0.01$, experimental method vs. conventional method. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, beginner vs. expert.

DISCUSSION:

The present study describes an easy-to-learn blood collection method in mice that is superior to the conventional techniques. First, the method can be easily mastered with a high success rate. Second, it is based on the vacuum negative pressure principle and allows for continuous drawing of blood with a reduced risk of direct blood exposure, which also reduces the chance of contamination and hemolysis[9]. Third, this method is feasible for frequent sampling of blood with adequate volumes from mice over a short period of time for various research purposes. Moreover, the procedure inflicts only minimal injury upon mice, and blood collection can be performed without the use of anesthetics; thus, the influence of the stress response and anesthetics on blood samples can be avoided.

The tail vein is a superior location for blood sampling according to the approved protocol[7]. However, it is not always easy to obtain sufficient blood volume from thin tail veins with low blood flows. In this case, the skin is usually cut open and vein is penetrated by a lancet, or the end of the tail is removed quickly by a razor.

This protocol aims to improve the methodology of blood collection from mice using the vacuum blood collection system, which requires a vacuum blood sample collection tube, butterfly needle,

and eyeglass magnifier. This vacuum blood sampling system is usually used for collecting blood samples from patients in daily clinical practice[10]. With the help of an eyeglass magnifier, the perfect puncture point of a tail vein is easier to locate. When the tip of a needle is inserted into the tail vein, blood will automatically flow into the vacuum tube due to negative pressure. After withdrawing the needle from the tail vein, the blood that is blocked in the catheter will flow into the collecting vacuum tube due to the vacuum.

The following tips are important for successful application of the method. First, the body weight of each mouse should be 40 g or higher to decrease difficulty in puncturing and obtaining enough blood. Second, in the case of failed blood collection, experimenters should try to withdraw the needle slowly until blood continues to flow out. Third, it is vital to extend the tail to avoid any movement during blood sampling. Holding the needle gently can help to keep the needle tip in the vein as the mouse tail moves. Fourth, if blood stops flowing out during blood collection, the needle angle should be adjusted in a timely manner. In order to avoid blood coagulation in the needle, another puncture position should be selected if blood stops flowing out after 15 s. Finally, the cooperation of two operators is recommended while using this technique to collect blood from mice.

In short, the adopted vacuum blood collection method for use in mice is safe, feasible, and easy to practice. This method enables frequent sampling of blood with adequate blood volumes from non-anesthetized mice.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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Figure 2

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Figure 3

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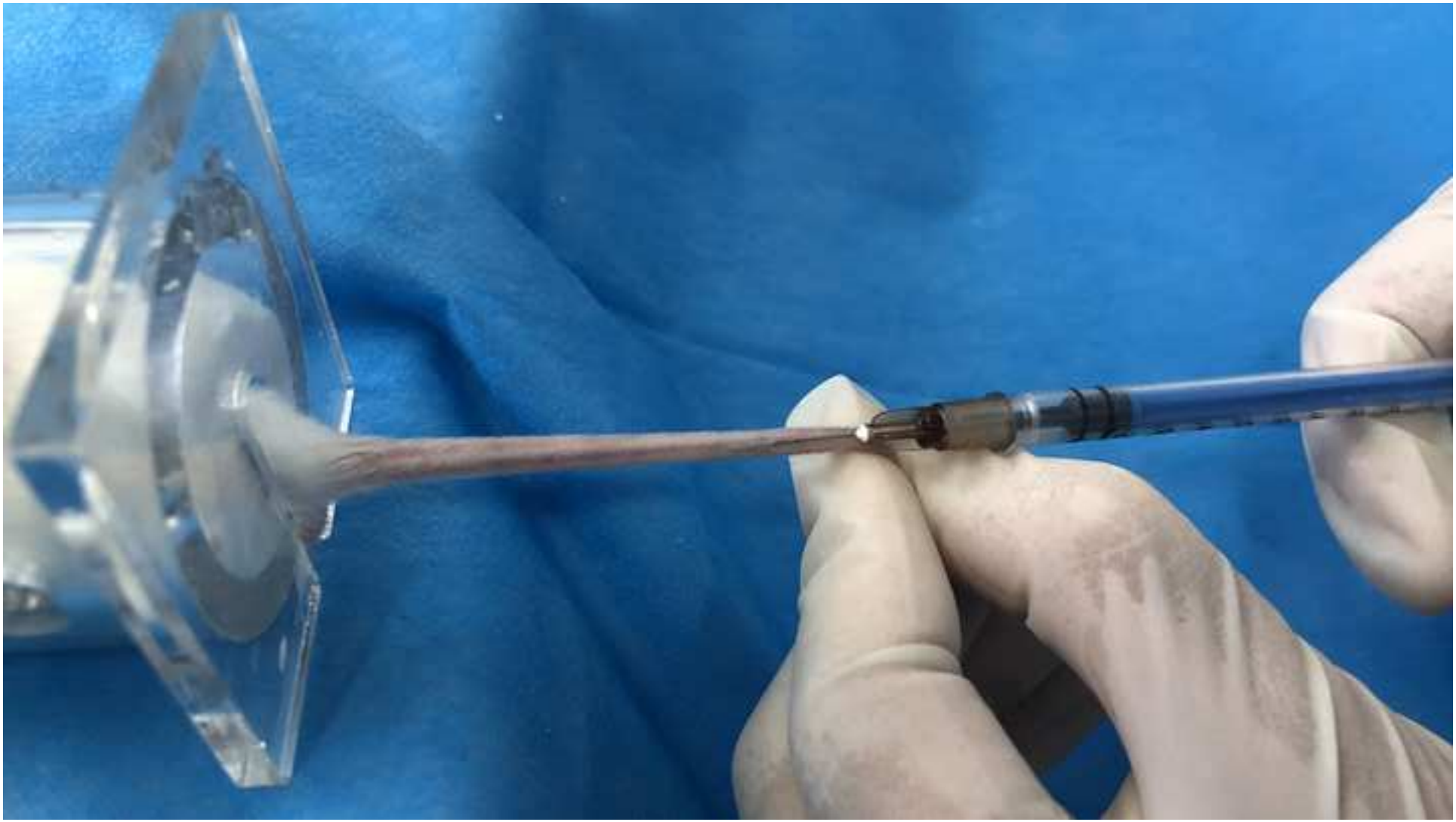

Table 1 Click here to access/download;Table;Table_1_122018.xlsx

	Experimental group	
	beginner	expert
Body mass (g)	42.4±1.42	42.55±2.91
Collected blood volume (μL)	172.00±15.17	184.25±11.95##
Sampling duration (min)	3.11±0.44	1.85±0.68##
Blood collection times	20	20
Average number of punctures	2.40±0.75	1.15±0.37##
One time puncture	3	17
Two times puncture	6	3
Three times puncture	5	0
Failed	6	0
Success rate	70%	100%##

Conventional group	
beginner	expert
42.65±1.14	43.20±2.69
148.50±30.22**	171.75±25.61#
4.08±0.61**	2.17±0.80##
20	20
2.90±0.31*	1.55±0.76##*
0	12
2	5
5	0
13	3
35%*	85%##

Table of Materials

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Name	Company	Catalogue number	Other details
Double-pointed needle	Shanghai Kang Nong medical instrument co., LTD, China	20163151718	22G (0.7 mm x 25 mm)
Eyeglass magnifier	Vergroberung		1.5x
Normal salt diet for mice			Mice received a normal salt diet (0.3% NaCl) throughout the study.
Plastic holder	Shanghai Kang Nong medical instrument co., LTD, China		35-45 g rat hoder
SPSS software for statistical analysis	SPSS Inc, Chicago; USA	Version 17.0.	
Syringe	Shandong wego Medical polymer products co. LTD., China	20160911A	1 mL (Matching needle size: 0.45×16RW LB)
Vacuum blood collection system	Wuhan Zhi Yuan, medical science and technology co., LTD, China	20171222	1 mL (Φ12.4×L75)

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Article Title: Modified Blood Collection from Tail Vein of Non-Anesthetized Mice with Vacuum Blood Collection System and Eyeglasses Magnifier

Signature: Wusong Zou Date: September. 13th. 2018

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JoVE59136 - [EMID:ee545e32a06ffc8f]

Dear Dr. Phillip Steindel

Thank you for giving us the chance to revise our manuscript, JoVE59136 "Modified Blood Collection from Tail Vein of Non-Anesthetized Mice with Vacuum Blood Collection System and Eyeglasses Magnifier,". We are grateful for the helpful comments from you and the reviewers. We carefully modified the manuscripts according related suggestions. Below are our point-to-point responses. We tracked the changes within the manuscript with blue marked text to identify all of the edits.

We also uploaded a separate rebuttal document that addressed each of the editorial and peer review comments individually.

Figures are submitted as psd files with 1920 x 1080 pixels.

Thanks for consideration.

Sincerely,
Wusong Zou, M.D. and Ye Gu M.D.

Editorial and production comments:
Changes to be made by the author(s) regarding the manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
We asked a native English speaker to help us to edit the manuscript now.

2. Table 1: Please note that the unit for sample duration is cut-off. Please format the table so all information shows correctly.
Done.

3. Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Information that can help readers to determine if the method is appropriate for their application.
Done (page:2, line:59-65).

4. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.
Done (page:3, line:91,97,100,128, page:4, line:137,138).

5. Please include a space between all numerical values and their corresponding units: 15 mL, 37 °C, 60 s; etc
Done (page:2, line:85, page:3, line:91,106,128, page:4, 153,154)

6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.
Changed accordingly (page:2, line:85,88, page:3, line:91).

7. Lines 96 and 97: Please write the text in the imperative tense in complete sentences. Are they placed in a sterile surface as stated in the video?
Sentence is modified, and the sterile condition is defined in the revised manuscript (page:3, line:97,98).

8. 2.6: Please specify exactly when the blood is collected.
The interval is 2 weeks, clarified in the revised manuscript (page:4, line:142).

9. Discussion: Please discuss any limitations of the technique.
Added (page:5, line:208-210;216-217).

10. References: Please do not abbreviate journal titles.
Changed (page:6, line:230,234,238,243,248,251,255,257).

11. Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Material/ Equipment.
Done.

Changes to be made by the Author(s) regarding the video:
1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.
We followed the suggestion and protocol and narration in the video is similar now（video: 02:02-02:53, page:3, line:100-110).

2. 02:24-02:29: Such details in the video are not mentioned in the written protocol. Please add them in step 2.2a. While step 2.2a starts with "Wear the eyeglasses magnifier...", the video does not show it.
We add "select the right or left tail vein for blood sampling. Grasp the tail in the lower portion near the tip gently and keep the tail tip straight during the sample collection." in the text（page:3, line:101-103)；"Wear the eyeglasses magnifier to improve the viewing to facilitate puncture of the tail vein." in the narration now（video: 02:24-02:30).

3. 06:23, 06:35: Please use the same figures for both the written manuscript and the video. For instance, the figure shown here is not included in the written manuscript.
Done（video: 06:15,06:38).

4. Please present the rest of the results in the video as well.
Done（video: 06:11-06:54).

5. Please upload a revised high-resolution video here: https://www.dropbox.com/request/RSpSe2PEA57w1rlSNexf
Done.

Audio issues
● The background music is competing a bit with the narration. The music volume should be lowered by about 6-9 dB.
Done.

Video quality issues

● **If the authors view the video on our website, they may notice that there is jagged artifacting on anything moving. This is because the video provided is interlaced. Our webplayer does not support interlacing**. **Future submissions should be provided at a progressive frame rate**. **When exporting, if there is a field where options for "Field Rendering" can be selected, "None" should be selected.**
Video file is modified according above requirements. Field Rendering selection option defined as None.

● **2:24, 2:31 - The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.**
We followed the suggestions and related modification is made now（video: 02:24,06:30).

● **2:58-3:40, 3:50-4:43 - This is a long time to go without any voiceover**. We understand the importance of seeing the blood slowly enter the tube, but do we need to see it in real time? We would recommend either fading to later in the shot, or adding narration that goes into more detail about what we are seeing.
We add more narration for overcome this phenomenon now（video:02:57-03:19, 03:24-03:40,03:46-04:50,04:52-05:10).

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The manuscript by Liu X et al describes an improvised method to collect blood samples from mouse. The methods described is simple and clear and the manuscript is well written.

Minor Concerns:
1. The English language needs to be improved throughout the manuscript. It will benefit the manuscript and the readers.
Thanks and a native English speaker helped us to modify the text during revision now.

2. In Table one, provide units for sampling duration.
Table one is deleted and a new table as table one is added.

3. In the materials chart include accurate details for the materials used such as Catalogue number. The details given under Catalogue numbers are inaccurate.
We followed the suggestions and related changes are made in the revised manuscript.

Reviewer #2:

Manuscript Summary:

The manuscript by Liu and colleagues describes a simple but efficient technique for improving blood collection process from mice through the tail-vein. The described protocol does not involve any sophisticated instrumentation or equipment and in-theory should be easy to implement.

Major Concerns:

The improvements in efficacy over a conventional procedure that does not use eyeglass magnifier and a vacuum system is marginal and therefore may not warrant publication. To make a stronger case for acceptance of this manuscript, it would be useful to have a larger number of data points (collection) by technicians of varying experience with the blood collection process.

Thanks and we followed your suggestion and related experiments are added, new results are presented and discussed in the revised manuscript now (page:4, line:156-162).

The primary purpose of this manuscript is not to introduce a novel technique but to describe it in a clear and effective manner for the readers to understand and implement. While the manuscript contains great details about the process, it is poorly written and prevents the reader from deriving useful information. It is therefore my recommendation that the manuscript be re-written with careful attention to language and grammar and could then be considered for publication.

Sorry for that and we asked a native English speaker to modify our manuscript now during the revision process and hope the typos are reduced to a minimal now.