# Journal of Visualized Experiments

## Sampling blood from the lateral tail vein of the rat

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | JoVE52766R2 |
| **Full Title:** | Sampling blood from the lateral tail vein of the rat |
| **Article Type:** | Invited Methods Article - JoVE Produced Video |
| **Keywords:** | Whole blood; catheter; minimally invasive; repeated sampling; plasma; serum; rat; neuroscience; endocrinology; stress |
| **Manuscript Classifications:** | 1.12.207.152: Blood; 2.1.50.150.900.649.865: Rodentia; 4.6.472: Hormones; 6.1.145.126.990: Stress, Psychological |
| **Corresponding Author:** | Ki Ann Goosens, Ph.D.<br>Massachusetts Institute of Technology<br>Cambridge, MA UNITED STATES |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author E-Mail:** | kgoosens@mit.edu |
| **Corresponding Author's Institution:** | Massachusetts Institute of Technology |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Graham Lee, Ph.D. |
| **First Author Secondary Information:** | |
| **Other Authors:** | Graham Lee, Ph.D. |
| **Order of Authors Secondary Information:** | |
| **Abstract:** | Blood samples are commonly obtained in many experimental contexts to measure targets of interest, including hormones, immune factors, growth factors, proteins, and glucose, yet the composition of the blood is dynamically regulated and easily perturbed. One factor that can change the blood composition is the stress response triggered by the sampling procedure, which can contribute to variability in the measures of interest. Here we describe a procedure for blood sampling from the lateral tail vein in the rat. This procedure offers significant advantages over other more commonly used techniques. It permits rapid sampling with minimal pain or invasiveness, without anesthesia or analgesia. Additionally, it can be used to obtain large volume samples (upwards of 1 ml in some rats), and it may be used repeatedly across experimental days. By minimizing the stress response and pain resulting from blood sampling, measures can more accurately reflect the true basal state of the animal, with minimal influence from the sampling procedure itself. |
| **Author Comments:** | Our manuscript is now fully revised in accordance with the reviewers' and editor's comments. |
| **Additional Information:** | |
| **Question** | **Response** |
| If this article needs to be "in-press" by a certain date to satisfy grant requirements, please indicate the date below and explain in your cover letter. | |
| If this article needs to be filmed by a certain date to due to author/equipment/lab availability, please indicate the date below and explain in your cover letter. | |

**TITLE:**
Sampling blood from the lateral tail vein of the rat

**AUTHORS:**
Lee, Graham
McGovern Institute for Brain Research
Massachusetts Institute of Technology
Cambridge, Massachusetts
gralee@mit.edu

Goosens, Ki A.
McGovern Institute for Brain Research, and the Department of Brain and Cognitive Sciences
Massachusetts Institute of Technology
Cambridge, Massachusetts
kgoosens@mit.edu

**CORRESPONDING AUTHOR:** Ki A. Goosens, Ph.D.

**KEYWORDS:**
Whole blood, catheter, minimally invasive, repeated sampling, plasma, serum, rat, neuroscience, endocrinology, stress

**SHORT ABSTRACT:**
Blood samples are useful for assessing biomarkers of physiological states or disease *in vivo*. Here we describe the methodology to sample blood from the lateral tail vein in the rat. This method provides rapid samples with minimal pain and invasiveness.

**LONG ABSTRACT:**
Blood samples are commonly obtained in many experimental contexts to measure targets of interest, including hormones, immune factors, growth factors, proteins, and glucose, yet the composition of the blood is dynamically regulated and easily perturbed. One factor that can change the blood composition is the stress response triggered by the sampling procedure, which can contribute to variability in the measures of interest. Here we describe a procedure for blood sampling from the lateral tail vein in the rat. This procedure offers significant advantages over other more commonly used techniques. It permits rapid sampling with minimal pain or invasiveness, without anesthesia or analgesia. Additionally, it can be used to obtain large volume samples (upwards of 1 ml in some rats), and it may be used repeatedly across experimental days. By minimizing the stress response and pain resulting from blood sampling, measures can more accurately reflect the true basal state of the animal, with minimal influence from the sampling procedure itself.

**INTRODUCTION:**

Biomarkers obtained from blood provide useful diagnostic, predictive, and stratifying measures in many clinical contexts, including cardiovascular disease [1], cancer sciences [2], and psychiatric disease [3]. They may also be used in basic science to assess the "state" of an organism, including the degree of hunger, inflammation, or stress present. Such measures can be influenced by variables that may or may not be critical to the question of interest, including the time of day that the sample is obtained and the gender of the subjects. It may also be influenced by the stress induced during the blood sampling procedures itself. Stress hormones and the perception of pain can rapidly alter the composition of the blood.

Rodents are the most commonly used laboratory animal, and multiple methods have been developed for blood collection. The ideal method of blood sampling should have minimal physiological impact on the animal, require no anesthesia, allow rapid and repeated sampling, and provide sufficient blood volume for numerous downstream applications. Popular techniques for collecting blood such as catheterization of the jugular vein or tail tip amputation do not meet these criteria.

The aim of this protocol is to describe a blood sampling technique for use in rats that is minimally stressful, does not require anesthesia, allows for multiple blood collections within a single subject, and provides a relatively large sample volume such that multiple assays may be performed on a single sample. The goal of this method is to obtain blood samples that are minimally influenced by the acute stress response.

**PROTOCOL:**

All experiments were done using adult male Long-Evans rats. All procedures were in accordance with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Massachusetts Institute of Technology and the Animal Care and Use Review Office of the USAMRMC.

**1. Preparation**

**1.1.** Heparinise the catheter and syringe by placing the shielded needle in a 500 µl tube containing heparin (1000 USP units/ml) and then aspirating and expelling heparin solution through the needle.

**1.1.1.** Attach a butterfly catheter to the syringe. Keep the shield over the needle of the catheter to protect the sharp tip from damage.

**1.1.2.** Withdraw a volume of heparin that is slightly greater than the volume of blood that will be collected. Detach the syringe and fill it with air.

**1.1.3.** Re-attach the syringe to the catheter and use the air to expel excess heparin solution; ensure only trace amounts remain in the tubing, needle, and syringe.

**1.1.4.** Place the sterile catheter, with the syringe still attached, on a sterile surface.

**1.2.** Quickly secure the rat in a clean cloth ensuring that fore- and hindpaws are in a comfortable position and breathing is unrestricted.

**1.2.1.** Secure the wrap with hook and loop tape; ensure that external genitalia are not constricted.

**1.2.2.** Have an assistant gently and firmly restrain the rat (abdomen and base of tail) on a solid work surface with the tail hanging off the edge of the counter.

**2. Blood Sampling**

**2.1.** Immerse the tail in 42 ˚C water for 40-50 sec to dilate blood vessels and dry the tail with a paper towel. Locate the tail vein to be bled (rotate the whole body with the tail to prevent twisting the tail).

Note: Sufficient warming of the tail is critical for the rapid collection of a blood sample. If the vasculature is constricted, proper placement of the catheter is difficult, and blood flow is vastly reduced. A heating pad may be used as an alternative to water immersion.

**2.2.** Identify the sampling point.

Note: The artery lies along the mid-dorsal aspect of the tail; do not use this for sampling.

**2.2.1.** Target either the left and right tail veins that lie lateral to the artery. Pigmentation of the tail, which varies by strain and increases with age, may obscure some of the vasculature. Target a portion of the vein in the lower portion of the tail.

**2.2.2.** Wipe the target area with 2% chlorhexidine antiseptic solution.

**2.3.** Create negative pressure in the syringe and catheter by withdrawing the plunger from zero to approximately 50 µl.

**2.4.** Hold the tail gently and firmly near the tip to keep the tail straight throughout sample collection. Ensure that blood flow is not occluded by an overly tight grip.

**2.5.** Slowly insert the catheter into the vein at a shallow angle approximately 5 cm from the tip of the tail. When the vein is penetrated, blood will flow into the catheter. Slowly withdraw the plunger of the syringe to collect the desired volume at a steady rate (~20 µl per sec).

**2.5.1.** Consult the veterinary staff for information about the maximum blood volume that can be collected. The maximum amount of blood that should be collected depends on the weight and health status of the rat. Do not withdraw more than 15% of total blood volume in a 14 day period.

Note: Blood is much more difficult to collect from animals that were acutely stressed in the minutes prior to sample collection because stress hormones constrict the vasculature. For example, moving the rat's home cage to a novel room, taking several minutes to wrap the animal, or repeated insertion of the catheter into a vein are all likely to trigger an acute stress response.

**2.5.2.** Facilitate blood flow by 'milking' the vein. Run a finger along the length of the vein, from the base towards the tip of the tail, but remain more than 2 cm from the tip of the inserted needle or the catheter may become dislodged from the vein.

**2.5.3.** If blood cannot be successfully collected from the initial site of catheter penetration, re-insert the needle further up the vein. If blood was collected at the initial site, re-pressurize the needle by disconnecting and then reconnecting the catheter and syringe prior to re-insertion in the vein. In general, avoid additional penetrations.

**2.5.4.** As multiple penetrations can cause tail vein collapse, in which the blood supply to the tail is cut off and the soft tail tissue becomes necrotized, euthanize the rat if there is tail vein collapse.

**2.6.** When adequate sample volume is collected, release pressure in the syringe by disconnecting and reconnecting the catheter. Aspirate slightly using the syringe plunger (~50 µl), and withdraw the needle from the vein.

Note: If the needle is withdrawn without first releasing the pressure in the syringe, blood will drip from the needle.

**2.7.** Briefly apply pressure to the insertion site to stop bleeding, and wipe the area with antiseptic solution. Return the rat to its home cage.

## 3. Processing the Blood Sample

**3.1.** Aspirate air to ensure no blood remains inside the catheter needle, and use scissors to cut the catheter tubing just above the needle. Expel the blood into a sterile 1.5 ml microcentrifuge tube.

Note: If blood is pushed through the needle, the shearing force may cause red blood cells to rupture which can interfere with many downstream assays. Remove the needle to avoid hemolysis.

**3.1.1.** To collect blood plasma, use tubes that contain EDTA as an anticoagulant (here, use 10 µl of 0.1 M EDTA for 200-400 µl of blood; ensure the concentration of EDTA used does not interfere with the downstream assay) and place on ice.

**3.1.1.1.** Spin whole blood samples at 2100 x g in a refrigerated centrifuge (4 ˚C) for 10 min within ten minutes of collection. Elute the plasma, avoiding disturbing the red and white blood cell layers.

**3.1.2.** To collect blood serum, place samples (without anticoagulant) at room temperature for up to 30 minutes to enable clotting. Spin the collection tubes in a refrigerated centrifuge (4˚C) at 2000 x g. The serum may then be eluted.

**3.2.** Use samples immediately, or store at -80 ˚C for up to one year.

**REPRESENTATIVE RESULTS:**

Blood plasma collected from the lateral tail vein as described in the protocol gives a plasma sample that was translucent and pale yellow in appearance. As shown in Figure 1, hemolysis in a sample imparts a red tint to the plasma. The acute stress response can rapidly alter the composition of blood. For example, circulating corticosterone concentration can markedly increase within 10 minutes of stressor exposure, as shown in Figure 2. The low basal levels of corticosterone obtained with this method prior to stressor exposure reveal that the sampling procedure itself is not a significant source of stress.

**Figure 1: Sample appearance.** A) A hemolyzed sample is shown. After centrifugation, the plasma or serum layer (surface indicated by the black arrow) appears tinged with pink or red. Darker tints indicate greater levels of hemolysis. B) After centrifugation, a properly collected sample will have a clear, yellowish appearance to the upper band (surface indicated by the black arrow), which corresponds to the non-hemolyzed plasma or serum. When removing this layer, it is important to not disturb the underlying whole blood, either by pushing the pipette tip into the whole blood layer or by aspirating some of the whole blood into the tip. Any plasma or serum contaminated with whole blood should be discarded.

**Figure 2: Plasma corticosterone is rapidly elevated following a stressful experience.** Blood was obtained from the lateral tail vein of adult female Long-Evans rats before and 10 minutes following exposure to 4 tones (10 sec, 2 kHz, 85 dB) co-terminating with footshocks (1 sec, 350 µA). Blood plasma corticosterone at baseline (290.4 ± 138.8 $pg.ml^{-1}$) was significantly less than the levels observed 10 minutes following presentation of the footshock stress (2204.8 ± 454.5 $pg.ml^{-1}$, $p = .02$, $n = 4$), as determined by paired t-test. *, $p < .05$

**DISCUSSION:**

Here, we describe a quick and simple procedure for obtaining a blood sample from a rat which offers significant advantages over other commonly used techniques. First, it does not require anesthesia, in contrast with sampling from the jugular vein or retroorbital sinus. When blood samples are collected surrounding behavioral procedures, administration of anesthetics is undesirable because it can interfere with learning and memory [4,5]. Second, it offers the ability to collect larger blood volumes than other venipuncture techniques, such as collection from the saphenous or dorsal pedal veins. Using the technique described here, up to 1.5 ml of blood may be collected from a rat at a single time point, a volume which readily allows multiple assays to be run in parallel. Finally, this procedure minimizes the potential for tissue damage compared to tail tip amputation or retroorbital bleeding. The use of this procedure facilitates compliance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*, which require minimizing the pain and distress that result from laboratory procedures performed on animals.

It is recommended that investigators new to this method practice the restraint and tail bleeding techniques in order to minimize the time that experimental animals are restrained. Blood collected in less than 3 minutes from the initiation of restraint provides optimal results.

The protocol described here may be used for sampling one to four times per week, but no more than twice per day. While repeated blood collections may be performed, different sampling sites moving upwards from the base of the tail should be used, and the left and right tail veins should be alternated as sampling sites. The total blood volume of rodents is 6-7% of their body weight, and no more than 15% of the total blood volume should be collected within a two week period. Serum or plasma comprises approximately 40-60% of the collected sample volume.

Blood sampling via the lateral tail veins may also be performed in the mouse as described here with a few minor modifications. First, only small gauge (27 g) catheters may be used. Second, it is recommended to use a tube restrainer, rather than a wrap, to immobilize the mice. The volume of blood that may be obtained from the mouse using venipuncture of the submandibular vascular bundle (200 – 500 µl) is greater than can be safely collected from the tail vein (200 µl maximum). Because sampling blood from the submandibular vascular bundle requires minimal restraint and may yield more blood, this is the preferred route for sampling in the mouse.

The rapidity with which this procedure may be performed, along with its minimally invasive nature, also minimizes the potential perturbation of blood-based measures by the acute stress response [6]. The acute stress response can alter circulating levels of many molecules, including interleukins and other immune-active factors [7], hormones of the hypothalamic-pituitary-adrenal axis [8], hormones in the sympathetic nervous system [9], ghrelin [10], endogenous opioids [11], dopamine, and serotonin [12]. If resting circulating measures of these molecules or others regulated by these molecules are desired, it is important to minimize the stress response, which is triggered within as little as a minute of the start of stressor exposure.

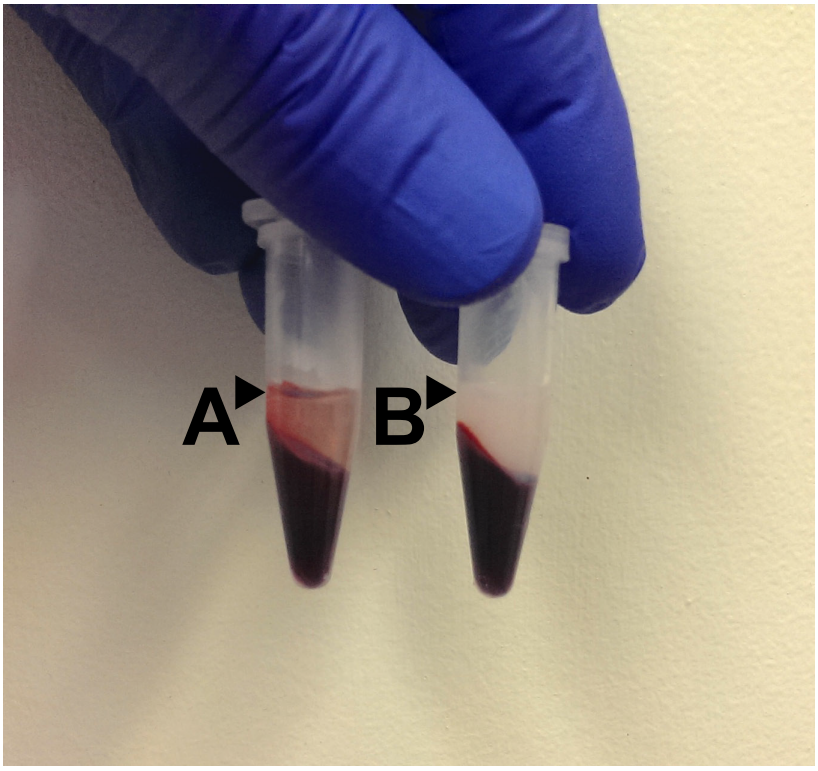
Stress responses not only alter the composition of the blood, but also represent a technical obstacle for blood sampling because of the constriction of vasculature via increased drive from the sympathetic nervous system. It becomes increasing difficult to obtain steady blood flow from a rat that is mounting an acute stress response. Therefore, the animal's distress must be minimized in order to rapidly obtain samples that reflect the physiological state of interest.

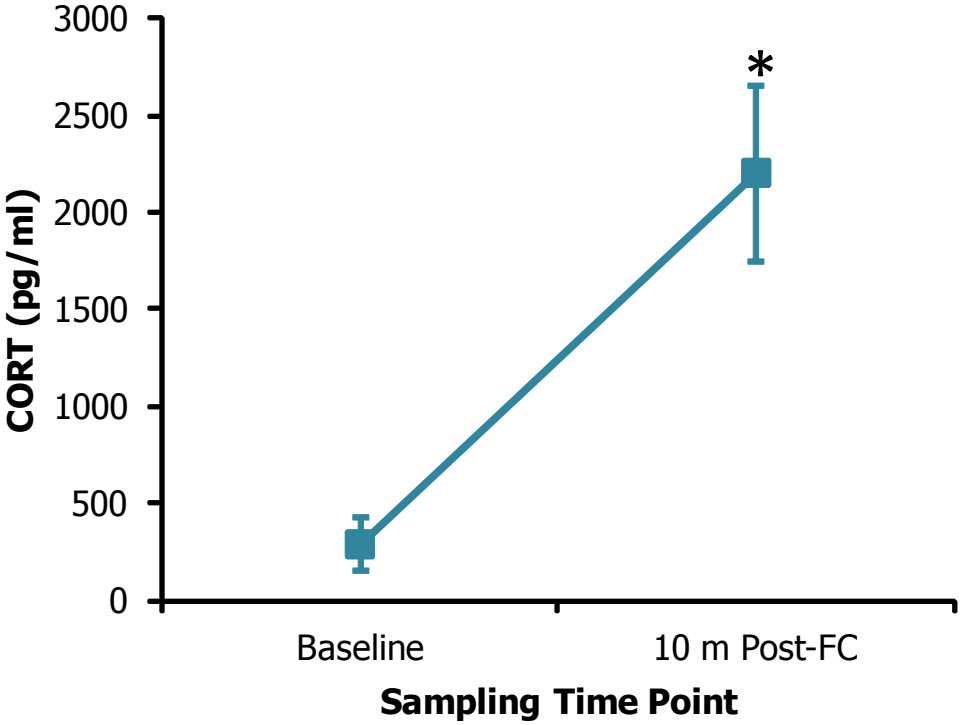
**DISCLOSURES:**
The authors have nothing to disclose.

**ACKNOWLEDGMENTS:**
We thank Virginia Doherty and Junmei Yao for technical assistance. This research was funded by NIMH (R01 MH084966), and the U.S. Army Research Office and the Defense Advanced Research Projects Agency (grant W911NF-10-1-0059) to KAG.

**REFERENCES**:
1 Vausort, M., Wagner, D. R. & Devaux, Y. Long Noncoding RNAs in Patients with Acute Myocardial Infarction. *Circ Res* **115** (7), 668-677, doi: 10.1161/CIRCRESAHA.115.303836 (2014).
2 Shah, R. *et al.* Biomarkers for Early Detection of Colorectal Cancer and Polyps: Systematic Review. *Cancer Epidemiol Biomarkers Prev* **23** (9), 1712-1728, doi: 10.1158/1055-9965.EPI-14-0412 (2014).
3 Chan, M. K. *et al.* Applications of blood-based protein biomarker strategies in the study of psychiatric disorders. *Prog Neurobiol*, doi: 10.1016/j.pneurobio.2014.08.002 (2014).
4 Cao, L., Li, L., Lin, D. & Zuo, Z. Isoflurane induces learning impairment that is mediated by interleukin 1beta in rodents. *PLoS One* **7** (12), e51431, doi: 10.1371/journal.pone.0051431 (2012).
5 Culley, D. J., Baxter, M. G., Yukhananov, R. & Crosby, G. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. *Anesthesiology* **100** (2), 309-314 (2004).
6 Vahl, T. P. *et al.* Comparative analysis of ACTH and corticosterone sampling methods in rats. *Am J Physiol Endocrinol Metab* **289** (5), E823-828, doi: 10.1152/ajpendo.00122.2005 (2005).
7 Kalinichenko, L. S., Koplik, E. V. & Pertsov, S. S. Cytokine profile of peripheral blood in rats with various behavioral characteristics during acute emotional stress. *Bull Exp Biol Med* **156** (4), 441-444, doi: 10.1007/s10517-014-2369-4 (2014).
8 McEwen, B. S. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol* **583** (2-3), 174-185, doi: 10.1016/j.ejphar.2007.11.071 (2008).
9 Sanchez, A., Toledo-Pinto, E. A., Menezes, M. L. & Pereira, O. C. Changes in norepinephrine and epinephrine concentrations in adrenal gland of the rats submitted to acute immobilization stress. *Pharmacol Res* **48** (6), 607-613 (2003).
10 Meyer, R. M., Burgos-Robles, A., Liu, E., Correia, S. S. & Goosens, K. A. A ghrelin-growth hormone axis drives stress-induced vulnerability to enhanced fear. *Mol Psychiatry*, doi:

mp2013135 [pii] 10.1038/mp.2013.135 (2013).
11 Knoll, A. T. & Carlezon, W. A., Jr. Dynorphin, stress, and depression. *Brain Res* **1314** (56-73), doi: 10.1016/j.brainres.2009.09.074 (2010).
12 Harvey, B. H., Brand, L., Jeeva, Z. & Stein, D. J. Cortical/hippocampal monoamines, HPA-axis changes and aversive behavior following stress and restress in an animal model of post-traumatic stress disorder. *Physiol Behav* **87** (5), 881-890, doi: 10.1016/j.physbeh.2006.01.033 (2006).


A
B


3000
2500
2000
1500
1000
500
0
CORT (pg/ml)
*
Baseline
10 m Post-FC
Sampling Time Point

Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment: JoVE_Materials Resubmit.xls

| Name of Material/ Equipment | Company | Catalog Number |
|---|---|---|
| Sodium heparin (1000 USP units/ml) | Patternson Veterinary Supply | 25021040010 |
| Ethylenediaminetetraacetic acid (EDTA) | JT Taylor | JT2020-01 |
| Dermachlor Rinse-Chlorhexadine | Butler Schein | 6356 |
| SURFLO Winged Infusion Sets, Terumo, butterfly catheters | VWR Scientific | TESV25BLK |
| BD Tuberculin 1cc syringes | VWR Scientific | BD309659 |
| 1.5 ml microcentrifuge tubes | VWR Scientific | 89202-682 |
| 500 µl microcentrifuge tubes | VWR Scientific | 21150-330 |
| Scissors, stainless steel, 5" | VWR Scientific | 82027-586 |
| 500ml plastic beaker | VWR Scientific | 414004-149 |
| Clean cloth wrap | Butler Schein | 2993 |
| Velcro tape, .75" width | Monoprice | B004AF9II6 |
| Timer | VWR Scientific | 62344-641 |

**Comments/Description**

Topical antiseptic solution, 2% chlorhexidine gluconate

Hook and loop tape

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

# ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Sampling blood from the lateral tail vein of the rat

Author(s): Graham Lee and Ki Goosens

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish ) via: ☐ Standard Access ☒ Open Access

Item 2 (check one box):

☒ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

## ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

# ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

**CORRESPONDING AUTHOR:**

| | |
|---|---|
| Name: | Ki Goosens |
| Department: | Brain and Cognitive Sciences |
| Institution: | Massachusetts Institute of Technology |
| Article Title: | Sampling blood from the lateral tail vein of the rat |
| Signature: | Ki Goosens |
| Date: | 9/30/14 |

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

**Response to Reviewers**

We thank the Science Editor and the reviews for their comments. We have now fully revised the manuscript in accordance with these suggestions.

**Science Editor Comments**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

   The manuscript has been reviewed by both authors for errors.

2. What is the concentration of the heparin solution used in step 1.1?

   The heparin concentration has now been added to the text.

3. What antiseptic solution is used in step 2.7?

   The antiseptic solution is now described in step 2.2.3.

4. Step 1.3 is the same as 1.2.

   Step 1.3 is now deleted.

5. Figure 1: A clearer tube would be better for depicting the results.

   We have now changed the picture in Figure 1 to more clearly illustrate the difference between the two tubes.

6. In addition to Figure 1, which shows the visual appearance of the blood sample, the authors should include at least one representative result that demonstrates the effectiveness of their technique and/or confirms sample viability with a quantitative measure. The abstract states, "The goal of this method is to obtain blood samples that are not altered by the acute stress response," so perhaps a representative result that compares data gathered using blood collected with this method to data collected using a sample that was altered by the acute stress response.

   As suggested by the editor, we have now added blood plasma corticosterone measurements to highlight the difference between pre-stress baseline and post-stressor exposure (Figure 2).

**Reviewer 1. Comments**

Major Concerns:

1. "…the manuscript could have a broader impact if some discussion was given as to how the protocol could be modified for blood collection in mouse."

   Blood collection from the tail vein is possible in the mouse, though sampling from the submandibular vascular bundle is the preferable method. A paragraph has been

**Response to Reviewers**

included in the discussion to reflect this and explain why submandibular bleeding is better for the mouse.

Minor Concerns

1. For Keywords, "three 'R's" is listed in the manuscript. I have no idea what that is.

The three 'R's, which refer to the federal requirement to reduce, replace and refine the use of laboratory animals, has been omitted.

2. For Introduction, consider rewording the last sentence to "The goal of this method is to minimize the influence of the acute stress response on obtaining the blood sample." Obviously the sampling procedure is still "stressful".

The last sentence in the introduction has been reworded to better describe the goal of the technique.

3. For Protocol, a heating pad might be an alternative tool for dilating blood vessels when a heated water source is unavailable (e.g. behavioral testing rooms).

This is an excellent suggestion. A heating pad as an alternative to heated water has been included in Step 2.1.1.

4. For Representative Results, figure subnumbering 1A and 1B are indicated in the text but A and B are not indicated on the figure.

Figure 1 is now modified to include the subcharacters to be consistent with the figure legend.

5. For Materials, consider adding product information for collection tubes and EDTA.

The EDTA and collection tube product information is now included in the materials list.

**Reviewer 2. Comments**

1. Two additional areas should be further addressed in the discussion. The authors discuss the importance of maintaining the rat in an unstressed state. The video will likely show this method at a slightly slower speed to allow the observers to study all the details. However, it is quite likely that this method must be completed relatively rapidly to obtain unstressed blood samples. The authors should describe a timeframe that would be optimal to obtain unstressed samples. Finally, it would also be useful is they could provide examples of corticosterone levels obtained using this procedure within an optimal time frame (compared to trunk blood of unstressed rats) to validate its use for obtaining unstressed blood samples.

An optimal time frame is now included in the discussion. We have also added data (Figure 2) that compare corticosterone levels taken in an "unstressed" state with those following an explicit stressor. These results show that the procedure performed in a "basal" state do not elevate corticosterone as does the stressor exposure.

2. 1.2 and 1.3 appear to be the same, so 1.3 could likely be deleted.

Step 1.3 has been deleted

**Response to Reviewers**

**Reviewer 3. Comments**

1. Are points 1.2 and 1.3 meant to be the same? Or is point 1.3 reaffirming that the rat should be secure after being moved to the edge of the table?

   Step 1.3 is now deleted. This was an accidental duplication on our part.