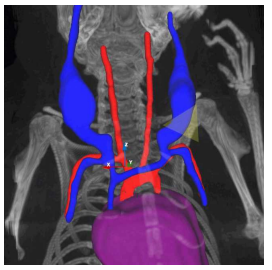
Aug 27, 2024

Sternoclavicular joint-Targeted External jugular venipuncture Method (STEM)



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Suguru Yamauchi[1,2,3], Andrei Gurau[1], Kaitlyn Ecoff[1], Kristen P. Rodgers[1], Yuping Mei[1], Frank Bosmans[4,5], Franck Housseau[6], Yun Chen[7], John Michel[6], Andreas S Barth[8], Jinny S Ha[9], Takumi Iwasawa[10], Kazunori Kato[10], Ryohma Tsuchiya[7], Miki Yamauchi[3], Hajime Orita[2,3], Shinji Mine[2], Tetsu Fukunaga[2], Malcolm V Brock[1]

[1]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA.;
[2]Department of Esophageal and Gastroenterological Surgery, Faculty of Medicine, Juntendo University, Tokyo, Japan.;
[3]International Collaborative Research Administration, Juntendo University School of Medicine, Tokyo, Japan.;
[4]Molecular Physiology and Neurophysics group, Department of Basic and Applied Medical Sciences, Faculty of Medicine and Public Health, University of Ghent, Belgium.;
[5]Experimental Pharmacology group (EFAR), Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, Vrije Universiteit Brussel, Belgium;
[6]Department of Oncology and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA.;
[7]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, USA.;
[8]Department of Medicine, Division of Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.;
[9]Division of Thoracic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA.;
[10]Institute of Life Innovation Studies, Toyo University, Tokyo, Japan.

Suguru Yamauchi
Johns Hopkins University

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Abstract

Vascular access in mice is a cornerstone of biomedical research, with peripheral venous approaches like the lateral tail vein, retrobulbar venous sinus, facial vein, and saphenous vein being common. However, central venous approaches are challenging due to animal size and required expertise. To address this, we developed the Sternoclavicular joint-Targeted External jugular veniMouse, Venipuncture, Blood collection, Blood sampling, Intravenous injection, Intravenous administration, Vascular access, Blood access, Central vein, Jugular vein, External jugular vein, Sternoclavicular jointpuncture Method (STEM). This technique provides reliable, longitudinal vascular access for frequent blood sampling using palpable surface anatomy landmarks. Moreover, STEM eliminates the need for fur shaving, specialized restraints, or deep sedation, allowing a single operator to perform the procedure safely and efficiently. Our protocol, based on a comprehensive anatomical analysis, revealed that the external jugular vein in mice traverses anteriorly to the clavicle before draining into the subclavian vein - a key anatomical difference from humans. This finding enabled a refined technique using the sternoclavicular joint as a landmark, improving the success and reproducibility of central venous access. Finally, STEM facilitates efficient blood collection and accurate intravenous administration with minimal setup time. It is straightforward and easily replicable, allowing researchers of all expertise levels to achieve high precision and reproducibility. The simplified learning process and consistent results make STEM valuable for various mouse-based experiments in biomedical research.

Guidelines

Animal Research:

- Reporting of *In Vivo* Experiments (ARRIVE) guidelines
- National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals

Materials

- Weight measurement device
- Powder-free exam gloves
- Swab stick Cotton Tip 3 inch
- 1ml Syringe with 26G x 3/8” needle
- 0.5 ml Syringe with 29G needle
- 0.3 ml Syringe with 29G needle
- Sharps Collector
- Blood Collection Tube

Reagents

- Ketamine
- Xylazine
- Chlorhexidine Solution

Reagent setup

Ketamine-xylazine cocktail

1.0 mL of Ketamine (100mg/mL) and 0.5 mL of Xylazine (20mg/mL) are diluted with 8.5 mL of saline and the final concentration is composed to [M] 10 mg/mL ketamine and [M] 1 mg/mL xylazine. A standard dose of this cocktail in mice is 0.1ml/10g. However, doses of 0.03ml/10g to 0.05ml/10g can still provide the anesthetic depth and safety required for this technique. Ketamine-xylazine cocktails should be stored in the refrigerator at 4 °C, away from light, and used within 30 days.

Before start

The maximum volume of blood that can be safely sampled from a mouse depends on several factors, including the mouse's body weight, the frequency of sampling, and whether fluid replacement is provided. For intravenous (IV) injection in mice, the total volume and rate of administration must be carefully considered for animal welfare and scientific validity. Rapid injection of large volumes can cause life-threatening complications such as pulmonary edema and significantly impact the cardiovascular, pulmonary, and renal systems, potentially confounding research outcomes. Be sure to follow the guidelines properly for both blood sampling and IV, and plan your experiments accordingly.

Procedure

1 **A. Blood sampling**

This original blood collection protocol for mice was developed for the left external jugular vein (EJV) and Fig 1 and **Video 1** showing the procedure in detail is provided.

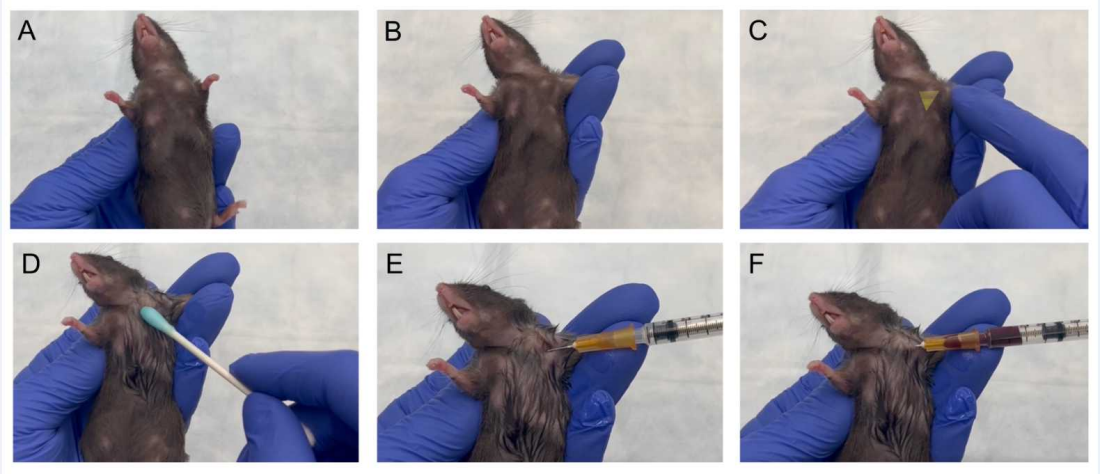

Fig 1

- We also share a modified version of this protocol that can be adapted for procedures involving the right EJV (Fig 2 and (**Video 2**).

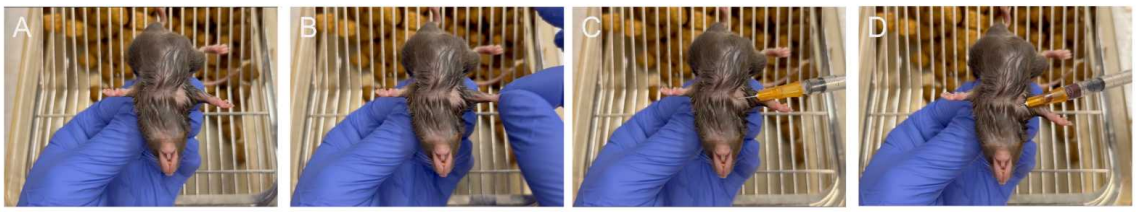

Fig 2

- This protocol primarily describes the procedure for blood collection from the left EJV.
- However, the accompanying high-resolution video documentation also delineates the procedural differences between accessing the left versus right EJV.
- These distinctions are crucial for researchers to consider when selecting the optimal site for venipuncture based on their specific experimental requirements.

Operator preparation

1m

2 Appropriately sized gloves should be worn.

Tips:

- Palpation of the skeletal muscles of the chest, the sternum, and clavicles is required as well as single-handed restraint of the mouse to ensure proper immobilization.

Anesthesia and mouse preparation

5m

3 **Tips:**

- We routinely use ketamine-xylazine for sedation and analgesia in mice.
- Ketamine-xylazine is a safe anesthetic with favorable sedative and muscle relaxant properties, and rapid resuscitation post procedure using antagonists is possible.
- Although inhaled anesthesia is routinely applied in mice, intraperitoneal administration was selected for this protocol due to its practicality in maintaining anesthesia throughout the procedure and to avoid potential interference from inhalation anesthesia equipment in the cervical region.

Note

- Alternative intraperitoneal anesthetic agents for mice may be substituted, with the exception of tribromoethanol, which should be used judiciously.
- Tribromoethanol, due to its documented potential as an irritant upon repeated administration and its propensity to induce intra-abdominal inflammation and adhesions, was excluded from consideration in this study for serial intraperitoneal anesthesia.

4 All mice should be weighed to determine the dose and schedule of anesthesia needed.

5 Secure the animal and disinfect the injection site before inserting the needle. The intraperitoneal injection site should be located laterally to the ventral midline in the caudal abdominal quadrant, either on the left or right side.

6 Advance the injection needle subcutaneously by about 5mm, then angle the needle vertically and proceed to penetrate the abdominal cavity.

7 Aspirate the syringe to confirm the absence of blood or intestinal fluid (indicated by brownish coloration), then inject a ketamine-xylazine cocktail (ketamine at 100 undetermined and xylazine at 10 undetermined and xyl) intraperitoneally using a 26G syringe needle.

8 Monitor the mouse closely during anesthesia induction.

9 Once anesthetized, transfer the mouse to the procedure area.

Tips:

- This procedure requires only minimal sedation, rather than full surgical anesthesia.
- Therefore, traditional assessments of anesthetic depth such as pedal withdrawal reflex, tail pinch, or abdominal skin pinch tests are not necessary.
- The goal is to achieve a level of sedation sufficient to prevent sudden movements that could interfere with the procedure.

Tips:

- The standard dose of the anesthetic cocktail is 0.1 mL per 10 g body weight.
- However, due to the rapid onset and reduced analgesic effect of this technique, one-third of the standard dose is often sufficient to achieve the desired anesthetic depth.

Note

While a lighter plane of anesthesia may facilitate faster recovery and enable earlier assessment of complications, deeper anesthesia can be administered when experimental protocols require minimizing animal stress.

Handling the mouse

2m

10 The mouse is restrained by grasping the back of the head or skin near the scapula between the thumb and index finger of the non-dominant hand, with the head immobilized between those fingers.

Tips:

- This allows dorsiflexion of the neck and widens the space between the clavicle and the intercostal space thus facilitating a thoracic approach with a needle (Fig 1A).

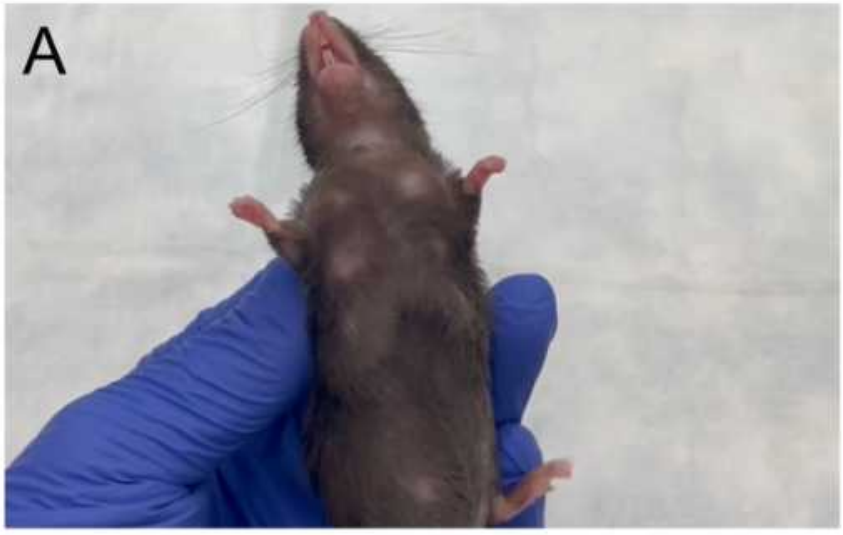

Fig 1A

- For right EJV puncture, grasp the mouse with the non-dominant hand by pinching a large fold of skin near the scapula.
- Position the mouse with its head tilted away from the operator to expose the right side of the neck (Fig 2A).

Fig 2A

11 The left front paw of the mouse should be externally rotated and restrained with the middle and ring fingers.

Note

Adequate outward tension on the skin is essential for clear visualization of the humero clavicular and sternoclavicular joints during the procedure (Fig 1B).

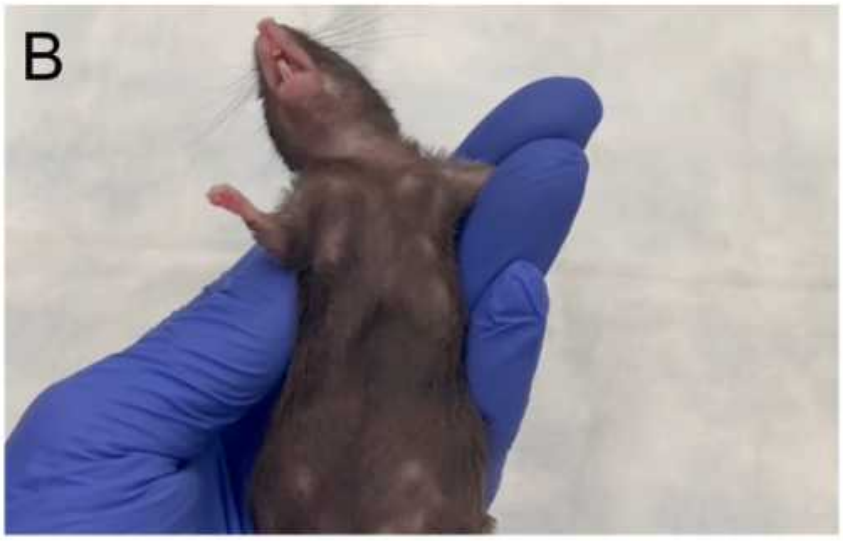

Fig 1B

12 The right front paw should also be abducted.

Tips:

- This procedure enhances the visual identification of the anatomical landmarks connecting the humerus, clavicle, and sternum through the skin surface.

Identify landmarks

3m

13 **Tips:**

- Precise identification of anatomical landmarks is crucial for the successful implementation of this novel venipuncture technique.
- Accurate recognition of landmarks significantly increases the likelihood of successful venous access.

The needle entry site is determined by identifying the triangular depression formed by the sternum and left clavicle at the sternoclavicular joint, which serves as a reliable anatomical landmark for venous access (Fig 1C).

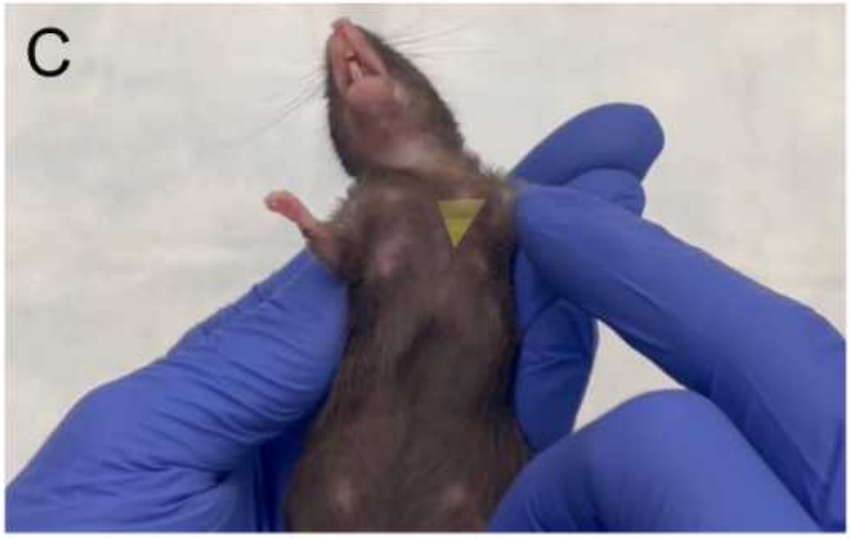

Fig 1C

Tips:

- The sternoclavicular joint, formed by the articulation of the sternum and clavicle, serves as the primary anatomical landmark due to its palpable prominence. The EJV courses superficially within the triangular region demarcated by this joint.

Disinfection

2m

14 The planned puncture site is disinfected three times with generously applied 0.2% chlorhexidine (Fig 1D).

Fig 1D

Tips:

- There is no need for fur removal.
- The disinfectant solution wets the fur, making it possible to visualize important anatomical structures.
- While applying disinfectant, careful palpation using cotton-tipped applicators with narrow tips can be performed to identify the precise location of relevant anatomical landmarks such as the sternum, clavicle, and sternoclavicular joint, as well as the triangular depression formed by these structures that serves as the target site for venipuncture.

Venipuncture

5m

15 **Tips:**

- Successful venipuncture is dependent on the precise location of needle entry and the angle and direction of needle advancement.

Note

The needle length was found to impact the ease of puncture. Needles longer than 1.27cm (0.5 inches) reduced tactile sensitivity at the needle tip, potentially compromising fine manipulation. Therefore, shorter needles were selected to maximize tactile feedback and improve control during the procedure.

The puncture site should be 2-3 mm lateral to the anatomical landmarks (Fig 1E and Fig 2C).

Fig 1E

Fig 2C

Tips:

- The puncture site is standardized at a point 5 mm lateral to the sternoclavicular joint along the subclavian border.
- This location was selected based on the assumption that the EJV has an approximate external width of 2 mm at its junction with the sternoclavicular joint.

16 The needle is advanced from the puncture site progressing along the subclavian border in a cranial direction.

Tips:

- Palpation of the subclavian border with the needle tip was used to verify the puncture position and standardize the procedure across mice, thereby minimizing inter-procedural variability.

17 While maintaining slight negative pressure on the syringe, the needle is advanced slowly in the direction of the previously identified anatomical marker.

Note

Although the inferior border of the clavicle serves as a reference for the puncture line, the needle is not advanced along the clavicle's longitudinal axis. This approach differs from typical subclavian venipuncture techniques. The EJV traverses the anterior aspect of the clavicle to reach the triangular target zone. It is important to note that the EJV is a superficial vessel. Shallow needle insertion avoids injury to underlying structures.

18 The needle should be slowly advanced into the vein while maintaining gentle negative pressure on the syringe plunger. Successful venipuncture and entry into the vascular lumen are confirmed by the appearance of blood in the syringe chamber.

Tips:

- If minimal blood return is observed in the syringe, suggesting partial vascular penetration, the needle is withdrawn 1-2 mm.
- Successful repositioning is confirmed by the resumption of consistent blood flow into the syringe chamber.

19 While maintaining the position of the mouse with the left hand, the operator can use the third and fourth digits to stabilize the syringe. Blood can then be carefully aspirated into the syringe while ensuring the needle remains stationary within the vessel lumen (Fig 1F and 2D).

Fig 1F

Fig 2D

Tips:

- To optimize blood collection, the needle tip should be securely positioned within the vascular lumen at a location where blood backflow can be visually confirmed.
- Blood should then be aspirated at a controlled rate to maintain vessel patency and minimize hemolysis.

Note

Rapid aspiration may induce venous collapse, interrupting blood flow. In such instances, a brief pause to allow for venous reperfusion often permits resumption of blood collection. Furthermore, brief, high-pressure aspirations can lead to hemolysis, potentially compromising subsequent hematological and biochemical analyses. Therefore, a controlled, steady aspiration technique is recommended to maintain sample integrity.

20 Once the required volume of blood is obtained, the needle is removed, and the blood can be transferred to the appropriate color-coded test tubes with or without additives.

Tips:

- Murine blood exhibits a high propensity for coagulation, necessitating rapid and appropriate anticoagulation measures to ensure sample integrity.
- The selection of anticoagulants is critical and can significantly influence subsequent hematological and biochemical analyses.

Postoperative management

3d

21 Hemostasis was achieved by applying direct pressure to the venipuncture site with a cotton swab for approximately 5 seconds.

Tips:

- Ensure that no signs of post-procedural bleeding are observed.

22 No further post-treatment is required, and the mouse is returned to its original cage.

23 Monitor the mouse closely during the recovery period to ensure that it is breathing normally and regaining consciousness.

24 Check the surgical site for any signs of bleeding, swelling, or infection.

Tips:

- Hemostasis typically occurs rapidly following venipuncture. Delayed hemorrhage is infrequently observed at the puncture site.

25 Post-procedural monitoring is conducted by:

(i) observing subject behavior and activity levels to assess return to baseline status;
(ii) checking food and water consumption to ensure normal intake patterns are maintained; and
(iii) performing health and behavioral assessments for several days to evaluate recovery progression.

Application for IV Injection

3d

26 **B. IV Injection**

Prepare a syringe and needle assembly with the necessary solution for IV administration. Steps 2 [go to step #2] through 18 [go to step #18] of the procedure are identical to those described for blood collection. The technique for IV injection into the EJV is demonstrated in **Video 3**. This IV injection method can be adapted for either the left or right EJV by modifying the mouse restraint technique accordingly.

Tips:

- For IV injections in mice, 0.3mL or 0.5mL syringes are preferable to 1mL syringes due to improved ergonomics and ease of single-handed manipulation.
- The use of smaller gauge needles, such as 29G, may facilitate more precise intravascular placement and infusion.
- Selection of syringe size should be based on hand dimensions of the operator to optimize control and reduce the risk of procedural errors.

Note

Prior to injection, all air must be meticulously evacuated from the syringe to prevent potentially fatal air embolism in the mouse.

27 Upon visual confirmation of blood backflow into the syringe, indicating proper intravascular placement, the plunger should be depressed slowly while monitoring for any resistance or extravasation of the injectate at the puncture site.

Tips:

- After confirming blood flashback, the needle tip is maintained in its position within the vessel for the duration of the injection procedure.
- To facilitate this technique, the dominant hand of the operator is used to adjust the grip and pressure on the syringe and plunger. Various syringe holding methods are shown (Fig 3).

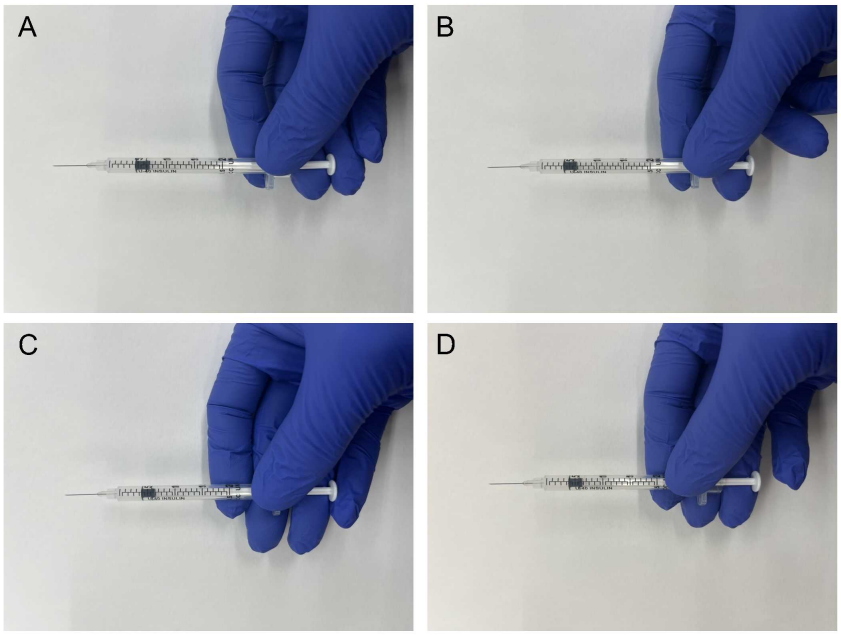

Fig 3

Tips:

- To verify intravascular needle placement throughout the injection procedure, intermittent aspiration is performed.
- The operator gently retracts the plunger at regular intervals during administration to confirm consistent blood return into the syringe hub.
- This technique ensures maintenance of needle tip position within the vessel lumen and complete intravascular delivery of the intended dose from initiation through completion of the bolus injection.

Note

Adhere to the maximum injection volume and injection rate to minimize the risk of acute complications such as volume overload or hemodynamic disturbances. Post-procedural monitoring is then conducted according to the protocol outlined in step 21 [go to step #21] of the experimental procedure.