

Perfusion Setup

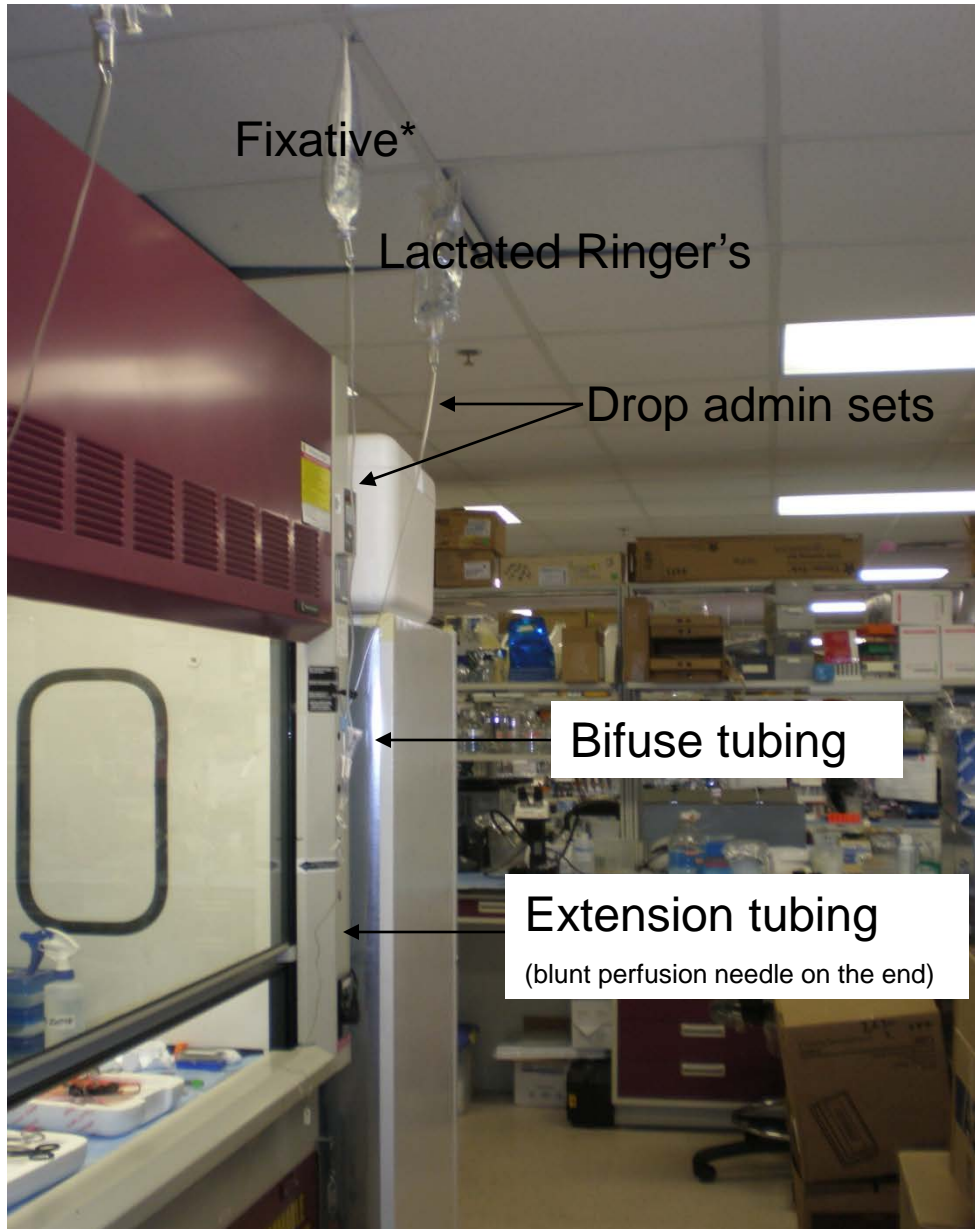
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Overall Setup



From Braun:

- Lactated Ringer's Injection USP, 1000mL, Cat#: L7500

From ICU Medical, Inc. (San Clemente, CA 92673):

- 41" 15 Drop Admin Set, Rotating Luer, Cat#: B3065
- 6" Smallbore Bifuse Ext Set w/ 3 clamps, Rotating Luer, Cat#: B1001

From DealMed Medical Supplies

<http://www.dealmed.com/Products/IV-Extension-Sets/60in-Small-Bore-IV-Extension-Set-Male-Female-Luer-Locks>):

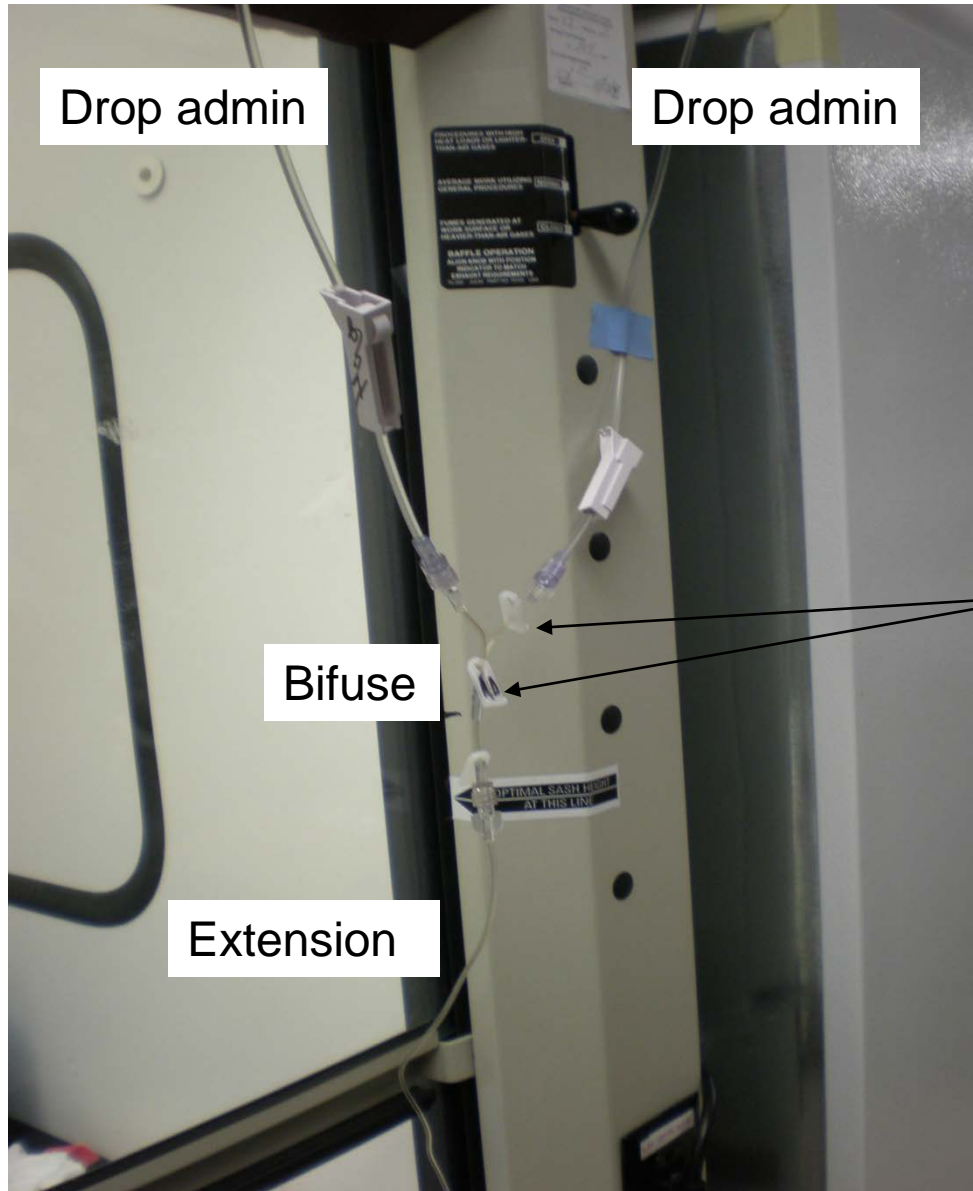
- 60" IV extension set
Male/Female Luer, 2.4 mL priming volume
Cat#: 398005

From Fine Science Tools:

- Blunt perfusion needle, Cat#: 18061-22 or -24

* We use empty lactated ringer's IV bags to hold the fixative, however, we recently found that the fixative sometimes reacts with the plastic bag (usually when the bag is reused more than 3 times).

Tubing



- Clamps are used to start and stop flow of lactated ringer's and fixative.
- Before beginning perfusions, make sure the lines are full and there no air bubbles – overall one drop admin tubing, and one “arm” of the bifuse tubing should be full of fixative, while the other drop admin tubing, the other “arm” of the bifuse tubing, AND the extension tubing should be full of lactated ringer's. The solutions should be at room temperature.

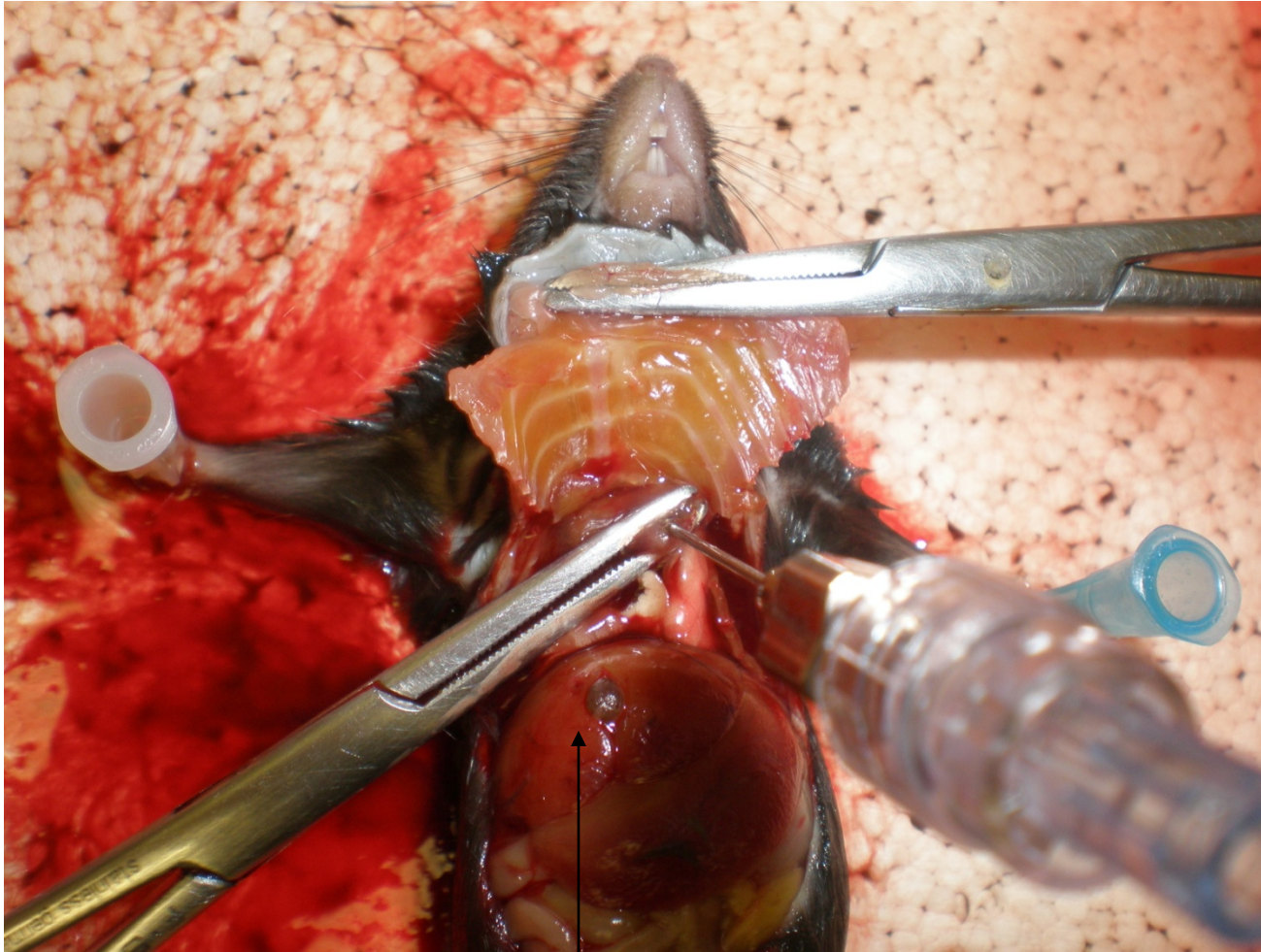
Needle into the heart



Mouse sedated with Isoflurane,
pinned to styrofoam

- 1.) Push the blunt perfusion needle into the left ventricle and clamp it in place with hemostat (“pinch up” some of the heart tissue around the needle to prevent back-flow – see next slide).
- 2.) Snip the right atrium with scissors
- 3.) Unclamp the lactated ringer’s clamp on the bifuse tubing for pre-rinse (~15 seconds), then re-clamp.
- 4.) Unclamp the fixative clamp on the bifuse tubing (~20 minutes).
- Note: We do not cut off the piece containing the sternum and ribcage because there is a big artery that runs through this area. We clamp it out of the way with a hemostat. We also like to expose the liver, as it is a good reference for perfusion quality (a liver that clears of blood = one sign of a good perfusion)

Close-up

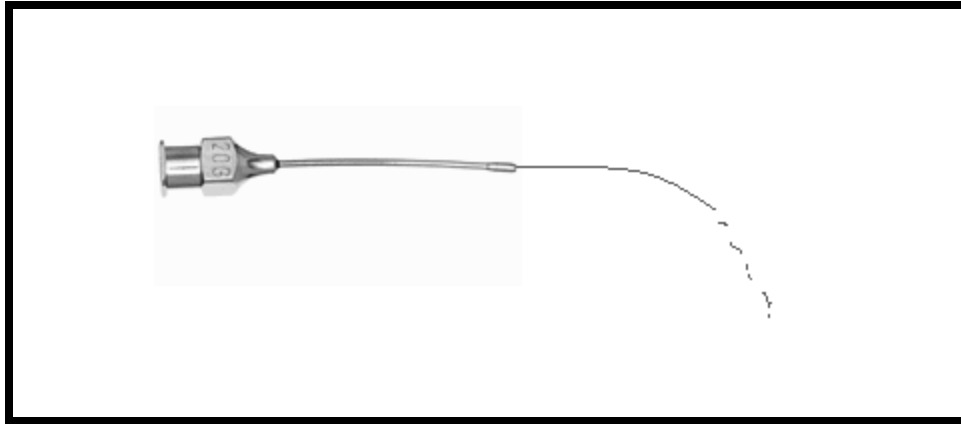


Note: The blunt perfusion needle is key!

When puncturing the heart with the needle, do so at the apex of the heart and stay as straight and to the left (mouse's left) as possible to avoid crossing the heart's septum (Overall, needle should run along parallel to the septum).

Uniformly clear liver (in this case it is very yellow due to using glutaraldehyde as the fixative).

Flow rate



The solution should be coming out of the needle at a decent rate. An arc of fluid, like in the image above, is usually good.

Fixatives:

- For IHC:
 - 4% paraformaldehyde dissolved in buffer (0.08 M sodium cacodylate or 1XPBS), pH 7.2- 7.4.
 - Low percentages of glutaraldehyde can be added to this solution (0.1% to 0.5%) to improve appearance of tissue, however this needs to be tested for each antibody used, as glutaraldehyde can adversely affect antigenicity.
- For good quality LM/EM:
 - 2.5% glutaraldehyde in 0.08 M sodium cacodyate + 0.05% calcium chloride, pH 7.2 -7.4
- For high quality LM/EM:
 - 5% glutaraldehyde in 0.08 M sodium cacodyate + 0.05% calcium chloride, pH 7.2 -7.4
- We typically use the first two and it is important to always make fresh fixative (right before use)!
- After perfusions, we dissect out our tissue of interest and immerse in the same fixative for 4 hours to overnight at 4°C with agitation.

Further Tissue Processing:

- After fixation, we wash with 0.1 M sodium cacodylate (or 1X PBS).
- If needed, we decalcify our tissues in 4.13% EDTA at 4°C with agitation (stir plate) for 2-4 weeks, depending on the age of the mouse.
 - 1 liter per pooled jaws or bones from 5 mice
 - Solution is changed ~every 2 days.
- After decalcifying we wash very thoroughly with the same wash above.
- Tissues are then processed for either paraffin, OCT (frozen), or plastic (epon, LR White).

Mouse Perfusion Supplies

Dr. Amelia Richardson
University of Michigan, Ann Arbor

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Blunt perfusion needle,
Cat#: 18061-22 or -24

From Fisher Scientific:

Sleeve rubber stoppers (e.g. Cat#: FB-578-80) that fit whatever 1L bottle you may have.