**Preparation and Take Down for Surgical Procedures**

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| ***Please provide a brief description of the type of work that this applies to (1 sentence MAX)*** |
| This describes the preoperative setup and postoperative take-down procedures for all surgical procedures. |

1. **Supplies:**
	1. Sterilized Surgical Tool Set in Autoclave Tray
		1. Scalpel handle (Fine Science Tools 10003-12 or equivalent)
		2. 45° forceps (Fine Science Tools 11251-35 or equivalent)
		3. Iris forceps (Fine Science Tools 11064-07 or equivalent)
		4. Black handle scissors, ToughCut (Fine Science Tools 14058-11 or equivalent)
		5. 45° Vanna scissors, 8cm (World Precision Instruments 500260 or equivalent)
		6. 45° or 90° Durotomy probe (Fine Science Tools 10032-13 or equivalent)
		7. Headpost
		8. Plastic sterilization container (Fine Science Tools 20810-02 or equivalent)
	2. Anesthesia
		1. Isoflurane (Piramal Critical Care Inc. 330250; purchased as Patterson Veterinary 07-890-8115 or equivalent)
	3. Materials **(Please note this section is a catch-all for all procedures, not all supplies and equipment are used for every surgical procedure. Refer to SOPs for complete list of required supplies for each specific procedure.)**
		1. Sterile Multi-well plate, 24 well (VWR 29443-952 or equivalent)
		2. Sterile Gauze, 3x3” squares, autoclave sterilized (Patterson Veterinary 07-847-3539 or equivalent)
		3. Gauze, 3x3” squares, non-sterile (Patterson Veterinary 07-847-3539 or equivalent)
		4. Cotton swabs, double ended, autoclave sterilized (VWR 89133-810 or equivalent)
		5. Cotton swabs, double ended, non-sterile (VWR 89133-810 or equivalent)
		6. Kimwipes, autoclave sterilized (VWR 21905-026 or equivalent)
		7. Kimwipes, non-sterile (VWR 21905-026 or equivalent)
		8. Sterile Surgical Drape, 18x26” (Moore Medical 14170 or equivalent)
		9. Press ‘n’ Seal (Amazon B001RHUP4Q or equivalent)
		10. Saran Wrap (Amazon B015FCLAVU or equivalent)
		11. Superglue, Krazy Glue, Precision Tip (Amazon B000BKO6DG or equivalent)
		12. Vetbond Glue (Patterson Veterinary 07-805-5031 or equivalent)
		13. Nair Hair Removal Cream (Amazon B00R4HWYNI or equivalent)
		14. Betadine solution (Moore Medical 77911 or equivalent)
		15. Gelfoam (Moore Medical 029828 or equivalent)
		16. Ortho-Jet BCA Powder (Lang Dental Ortho-Jet™ Powder or equivalent)
		17. Black Tempura Paint (Sargent Art 22-7185 or equivalent)
		18. Ortho-Jet BCA Liquid (Lang Dental Ortho-Jet BCA Liquid or equivalent)
		19. Coverslip Assembly (Tower Optical custom made)
		20. 7mm Single Coverslip (Warner Instruments 64-0701 or equivalent)
		21. O-Ring Well (Dygert 1RJG8 OR Dygert 1KET7 or equivalent)
		22. Sugi pointed sterile swabs (Kettenbach 3061 or equivalent)
		23. Alcohol Wipes (VWR 15648-907 or equivalent)
		24. Insulin syringes, U-100, 0.3 ml, 31G (VWR BD328438 or equivalent)
		25. Insulin syringes, U-100, 1 ml, 31G (VWR BDBD328418 or equivalent)
		26. C Universal 4-META Catalyst, 0.7 ml (Parkell S371 or equivalent)
		27. B Quick Base for MetaBond, 10 ml (Parkell S398 or equivalent)
		28. Radiopaque L-Powder, white, 5 gm (Parkell S396 or equivalent)
		29. Radiopaque L-Powder, clear 3 gm (Parkell S399 or equivalent)
		30. Puralube Veterinary Ointment (Dechra 17033-211-30 or equivalent)
		31. PREempt Disinfectant spray (Contecinc 21101 or equivalent)
		32. 70% Ethanol spray bottle (RP0032 or eqivalent)
		33. Artificial Cerebrospinal Fluid.V (RP0205 or equivalent)
		34. Luer-Lock Syringe, 20 ml (VWR BD 309661 or equivalent)
		35. Luer-Lock Syringe, 10ml (VWR BD309604 or equivalent)
		36. 25G 5/8 inch needle (VWR BD305122 or equivalent)
		37. 32mm Syringe Filter .2um Supor Membrane (VWR 28143-350 or equivalent)
		38. 3 ml transfer pipette, plastic (VWR 52947-970 or equivalent)
		39. Sterilization pouches (Chex-All 082411 or equivalent)
		40. Sterile Drill Bits, 0.5/0.4 (NeoBurr FG1/4 or equivalent)
		41. Sterile Drill Bits, 1.4/1.1 (NeoBurr FG4 or equivalent)
		42. Sterile Drill Bits 1.0/4.2 (NeoBurr EF4 or equivalent)
		43. Sterile Scalpel blades, #10 (World Precision Instruments 500239 or equivalentt)
		44. Sterile Scalpel blades, #11 (World Precision Instruments 500240 or equivalent)
		45. Non-Sterile Gloves (Microflex Ultraform Gloves or equivalent)
		46. Kwik Cast (World Precision Instruments Kwik-Cast or equivalent)
		47. 5-0 Monofilament suture with 17mm 1/2C taper needle attached, needle
		48. Heat-sterilized Glass pipettes (Drummond Scientific 3-000-203-G/X, World Precision Instruments 1B120F-4 or equivalent)
		49. “Marker” glass pipette, pulled, broken, and Sharpie for measuring coordinates (World Precision Instruments 1B120F-4 or equivalent))
		50. Microfil (World Precision Instruments, MF28G67-5)
		51. Sterile Bone Wax (Lukens 901)
		52. Lightweight Mineral Oil (Sigma Aldrich M8410 or equivalent)
		53. 30 gauge, 2" Backfilling Needle (Amazon B00EOAI5HC or equivalent)
	4. Equipment
		1. Bead sterilizer (Germinator 500 or equivalent)
		2. Dental Drill (NSKPana or equivalent)
		3. Stereo Microscope (Leica M80 or equivalent)
		4. Small Animal Stereotaxic Instrument (Kopf 1900 or equivalent)
		5. Small Animal Temperature Control System (CWE Inc. TC-1000 or equivalent)
		6. Fiber optic illuminator (Dolan Jenner MI-152 or equivalent)
		7. Isoflurane with oxygen delivery system (Patterson Scientific 07-8914722 or equivalent)
		8. Oxygen Concentrator (Puraline or equivalent)
		9. Isoflurane induction chamber (Patterson Scientific 078917853 or equivalent)
		10. Heat plate/pad (Lectro Kennel Outdoor Heating Pad or equivalent)
		11. Digital calipers (Amazon 784EC or equivalent)
		12. Galaxy Mini Centrifuge (VWRC1413V-230 EU or equivalent)
		13. Pipette Puller (Sutter Instrument P-97 or equivalent)
		14. Electrode Holder (Kopf 1970 or equivalent)
		15. Midgard Precision Current Source (Stoelting 51595 or equivalent)
		16. Silver wire (Stoelting 50880 or equivalent)
		17. P20 Pipettor (Gilson Inc F123600 or equivalent)
		18. Nanoject II Variable Volume (2.3 to 69 nL) Automatic Injector (VWR 490007-164 or equivalent)
2. **Personal Protective Procedures (PPE):**
	* 1. Non-Sterile Gloves
		2. Disposable lab coat
		3. Face mask; 0.6 micron filter (Optional)
		4. Shoe covers or designated surgery shoes
		5. Scrubs
		6. Surgical Cap or hair secured
		7. Biohazard sharps disposal container
		8. Biohazard waste disposal container
3. **Setup for all Procedures:**
	1. Please note that these steps can be performed out of order at the surgeon’s discretion unless otherwise indicated, except sterile area should be completely prepared and all sterile supplies arranged before animal is prepared for surgery. Animal preparation should be the last step before surgery.
	2. Remove all hand jewelry and wash hands thoroughly with soap and water. Don exam gloves and spray with alcohol. Gloves should be changed any time they become damaged, after each animal is prepared for surgery, or if gloves become grossly contaminated during the surgery. Gloves should be sprayed with alcohol if surgeon touches non-sterile items, before handling sterilized items. Gloves should be changed between surgeries.
	3. **ATTENTION: Before proceeding, please reference the “**[**Standards of Care Protocol**](http://aibsfileprint/legal/RegCentral/IACUC/Lists/Protocols/Attachments/68/Standard%20of%20Care%20Protocol%20Amendment%202_%282-7-17%29.xlsx)**.xlsx” for complete list of drugs and drug preparations required by the procedure being performed**
	4. Turn on microscope lights, bead sterilizer (if performing multiple surgeries) and heating pad. Set the heating pad to 37.0 ± 0.5°C.
	5. Disinfect the surgery area.
		1. Spray down the surgery area with disinfectant spray cleaner and wait 5 minutes before wiping the surfaces with a kimwipe. Wipe down surgical rig with alcohol, being sure to avoid getting alcohol in the threads of the instrument. Wipe down the heating pad, nose cone, and bite bar with alcohol to disinfect.
		2. Wipe down with alcohol light casings, stereotaxic knobs, and surfaces of the microscope which will be touched during surgery
		3. Wipe down drill and drill stand with alcohol. If performing multiple surgeries, repeat this step between procedures.
		4. Clean out isoflurane induction chamber with disinfectant spray. If performing multiple surgeries, repeat this step between procedures.
	6. Prepare the materials required for the surgery.
		1. Spray gloved hands with alcohol.
		2. Touching only the blue side to ensure sterility, open a sterile drape and place it, white side up and blue side down, on recently disinfected area.
		3. Open sterile packages of cotton swabs, kimwipes, gauze. Without touching the supplies with your hands, drop the items onto the surgical drape to preserve sterility.
		4. Remove surgery tools from the sterilization tray and place on the sterile drape, taking care to not touch the instrument tips.
		5. Remove a well from the designated jar or pack, spray with 70% Ethanol and place on the sterile drape (only if required by surgery type)
		6. Cover heating pad on surgical rig with a layer of press ‘n’ seal.
		7. Open sterile 24-well plate and dispense supplies into well
		8. Fill the 10 or 20 ml syringe with sterile Artificial Cerebrospinal Fluid (ACSF), maintaining the sterility of the solution and the syringe
		9. Attach a 25G 5/8” needle on a sterile 0.2 μm syringe filter and attach the 10 or 20 ml syringe of ACSF to the filter. NOTE: Never pull fluid through needle into the filter, only expel from the syringe through the filter.
		10. Place on sterile drape. Note: sterile saline is an acceptable substitute for practice surgeries and training, however, ACSF is always preferable.
4. **Setup Procedures specific to HP Transcranial, HP Ephys, and HP+C**
	1. Fill one well with 1 ml Nair.
	2. Fill one well with 1 ml Betadine solution and soak three swabs
	3. Fill three wells with black cement powder (premixed at a ratio of 4:1 cement to paint) (only if required by surgery type)
	4. Use sterile forceps to tear off pieces of sterile Gelfoam, and place in one well. The number of pieces of Gelfoam will vary depending on each surgeon’s preference. (only if performing HP+C)
	5. Using forceps to prevent touching the coverslip with gloves, place one in a well and cover with 70% Ethanol to soak.
	6. Fill the well containing Gelfoam with ACSF to soak prior to use.
	7. Fill one or two wells with ACSF.
	8. Disinfect drill with 70% Ethanol.Making sure to maintain sterility of the drill bit, attach the drill bit to the drill
5. **Setup procedures for Iontophoretic Injection (with or without headpost)**
	1. Obtain 1.2 mm diameter glass pipette, already pulled with the tip already broken (stored in plastic boxes in surgical suite).
		1. Set aside and only fill pipette with virus imeediately (reference proper SOP for detailed instructions) before injection to prevent clogging the pipette
	2. Remove one aliquot of virus from -80ºC freezer, thaw at room temperature, and spin down in the mini centrifuge, and store in wet ice
	3. Fill one well of a 24-well plate with Betadine and a second well with Nair. Place on the surgical drape (for injection on non-headposted animal)
	4. After mouse has been anesthetized in the induction chamber (see instructions above), place animal on the surgical rig, either in the ear bars or in specified clamp based on procedure type.
6. **Setup procedures for Nanoject Injection (with or without headpost)**
	1. Obtain Nanoject-specific pipette, already pulled and broken at the proper distance from the shoulder.
	2. Remove one aliquot of virus from -80ºC freezer, thaw at room temperature, and spin down in the mini centrifuge, and store in wet ice
	3. Fill one well of a 24-well plate with Betadine and a second well with Nair. Place on the surgical drape (for injection on non-headposted animal)
	4. Prepare pipette
		1. Using a 30g, 2” backfilling Hamilton syringe filled with mineral oil, backfill the pipette
		2. Insert the tip of the Hamilton syringe into the pulled pipette, all the way to the shoulder, and slowly depress the plunger on the Hamilton syringe, filling the pipette with oil. Be sure not to introduce bubbles into the system or the injection may not be successful.
		3. Once the pipette is backfilled with oil, loosen the collet on the end of the injector (see Figure 1).
		4. The pointed wire plunger should be positioned to just see the tip flush with the end of the collet (slightly recessed is also acceptable). This is called the “home position”. It is critical to put it back in the home position to protect the wire plunger from damage when finished working with the Nanoject.
		5. Insert the blunt end of the pipette through the front of the collet and the o-ring with the large hole.

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| **Figure 1:** Pipette with collet and o-ring. |

* + 1. Push the blunt end of the pipette onto the wire plunger and as the tip is pushed on, feel it go through the large O-ring and seat in the white spacer.
		2. Once positioned, tighten the collet securely. *Gently* pull on the pipette to confirm it is securely mounted.

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| **Figure 2**: Injection Standard Collet/O-Ring.**Source:** <https://www.drummondsci.com/wp-content/uploads/2019/10/Nanoject-II-Manual.pdf> |

* 1. Load the virus into the pipette.
		1. Once the pipette is secured to the collet, press and hold the ‘EMPTY’ button on the control box. This will drive the wire plunger out forcing oil to the tip of the pipette. Any excess oil will be expelled. Expel about 4-5 drops and work out any air bubbles. Note: Do NOT extend the plunger to its full length. This can throw off the calibration of the Nanoject and lead to inaccurate injection volumes.
		2. Take virus from ice and spin down for 10-15 seconds.
		3. Use the P20 micropipette with a microfil tip to draw up ~2 µl of virus.
		4. Using the surgical bed as a platform, aspirate the virus sample onto a clean piece of Parafilm.

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| **Figure 3:** Loading virus into Nanoject. |

* + 1. Using the stereotaxic apparatus, lower the tip of the pipette (secured in the Nanoject) into the sample. Be careful to not “bottom out”.
		2. Press and hold the ‘FILL’ button and draw up the desired amount of virus. Release the button when finished.
		3. Do not introduce air bubbles into the system as this may result in inaccurate injection volumes.
	1. Use standard injection volume of 50.6 nl per press at the slow rate of 23 nl/sec and repeat to reach target injection volume. Volume switches 1-5 should be in the D-D-U-D-D (----) positions, respectively. (unless otherwise specified by surgical work request)

**NOTE:** Injection volumes are determined by the position of the dip switches on the side of the control box. Switches 1-4 control the volume, while the fifth switch controls the injection and fill rates.

* + 1. Once the volume has been selected, each time the ‘INJECT’ button is depressed an audible beep will be heard and the selected volume will be dispensed.
		2. Multiple injections can be made in one location by simply pressing “inject" again. If "inject" is pressed again before the first injection is complete, it will not produce a second injection.
	1. Carefully set injector aside and prepare animal for surgery.
	2. After mouse has been anesthetized in the induction chamber (see instructions above), place animal on the surgical rig, either in the ear bars or designated clamp.
	3. Prepare the Anesthesia system
		1. Ensure the vacuum gauge is set to 10-15 pounds per square inch (PSI) and that all vacuum lines are functioning correctly for both the induction chamber and nosecone scoop.
		2. Connect induction chamber to the Isoflurane and Oxygen.
		3. Turn the oxygen concentrator on if applicable.
		4. Ensure the Oxygen regulator is set to 0.8-1.0 (0.8-1 L/min).
		5. Double check all the gas tubing to ensure the system is connected correctly.
	4. Anaesthetize the mouse.
		1. Open the vacuum valve and Isoflurane valves. Direct the flow to the induction chamber.
		2. Remove the animal from it’s experimental cage, obtain a preoperative weight and record on surgical work request, along with cage number, sex, and birthdate.
		3. Place the mouse into the induction chamber.
		4. Turn the Isoflurane regulator to setting 5.0 (5%). Note the time of beginning Isoflurane administration on the surgical work request form.
		5. Once the mouse is fully unconscious, turn off the Isoflurane. Leave the oxygen and vacuum lines open while removing the mouse from the induction chamber.

**NOTE:** When the animal is not moving and is having about one breath every 3 seconds the animal should be safe to transfer to the nose-cone

* + 1. Position mouse on surgical rig, either with ear bars or with specified clamping mechanism (see sections below for more detail)
		2. Secure the nose cone over the mouse’s snout. Make sure the body of the mouse is on top of the heading pad, resting comfortably. Redirect the gas flow from the induction chamber to the surgical rig.
		3. Set the Isoflurane to ~1.5-2%.
		4. Turn off the vacuum line of the induction chamber and close the lid.
		5. Monitor the mouse’s breathing throughout the process and adjust gas levels as necessary.

**NOTE:** Check if the animal responds to a toe pinch, if the animal responds, increase the Isoflurane level

**NOTE:** If the breathing is rapid and shallow increase the Isoflurane level.

**NOTE:** If the breathing is too deep, decrease the Isoflurane level.

* 1. Prepare the mouse for surgery.
		1. Apply Puralube to the end of a cotton swab and use to push the whiskers away from the surgical field.
		2. Cover the mouse’s eyes with a generous amount of Puralube. Additional Puralube should be added as needed to prevent eye dryness.
	2. After animal has been anesthetized in the induction chamber, secure the ear bars into the ear canals or the temple just outside the ear cavity of the mouse. Check to make sure the head is straight and symmetrical and that the ear bars are extremely secure. The head should not move when touched
	3. Use non-sterile scissors to shorten hair from top of the head and left side of the face. Hair should be removed to behind the ears and as far forwards at the between the eyes. Do not remove hair all the way down the snout of the animal. Use caution when trimming hair around the eyes. When finished with the scissors, wipe scissors with alcohol, but do not put them back on the sterile drape. These scissors should be reserved for cutting the hair only.
	4. Remove remaining hair with Nair. Apply the Nair with the pointed end of a non-sterile cotton swab. Remove Nair within 25 seconds of application.

**NOTE:** Some animalswill not responding to the toe pinch but will react to the nair. In this instance increase the Isoflurane and closely monitor breathing.

**NOTE:** If the issue persists ask a senior surgeon for help.

* 1. Remove all Nair with several clean, non-sterile cotton swabs or alcohol wipes. Use each swab for only one swipe. Wipe down the head surface with ACSF or alcohol wipes and clean up with kimwipes or cotton swabs to prevent Nair burns
	2. Remove loose hair and disinfect head with 3 rounds of alternating Betadine-soaked sterile swabs and alcohol wipes. The last application of Betadine should not be wiped off.
	3. Remove open gauze (hair catch) from underneath mouse and discard in biohazard bin.
	4. Change gloves.
	5. Cover the body of the mouse up to the surgical site with saran wrap leaving the head
	6. uncovered, being careful to not touch the mouse.
1. **General principles for “tips only” aseptic surgery**
	1. Do not touch the surgical site with hands after initial incision is made.
	2. All items that come into contact must be sterile to the maximum extent possible.
	3. Restrilize tips of surgical tools after initial incision and any time the tips touch a non-sterile surface.
	4. A new suture pack should be used for every animal. Gloves should be changed or disinfected before handling suture material.
2. **Between surgeries**
	1. After animal is removed from the rig and all immediate post-operative animal care steps have been completed, change gloves.
	2. Disinfect the surgery area as described in step 3.6
	3. Resterilize tips of all surgical insturments.
3. **Take down Procedure**
	1. Dispose of used syringes, blades, drill bits and needles in the biohazard sharps container.
	2. Dispose of all disposable materials that came into contact with blood in the biohazard waste container.
	3. Spray down surgical tools with **pH neutral surgical tool cleaner and wipe with a kimwipe or alcohol swab. Be sure to** wipe any blood, skin or cement residue off of the tools. Use caution when wiping down #5 dumonts and 45° forceps. Wrap with autoclave pack and write appropriate department initials and the date on the pack. Bring the tray to the designated location to be autoclaved.
		1. If the tools are to be used again the same day, sterilize using the hot bead dry sterilizer.
	4. Disinfect ear bars with 70% Ethanol, then place back on the surgical rig.
	5. Release the air from the drill lines by pressing down on the pedals until the pressure reads 0 psi. If the air source is connected to a wall valve, then turn the valve to the off position.
	6. Turn off the vacuum and oxygen sources by switching the stopcocks to the off position.