

## Flexivent Protocol

Updated 6-13-19

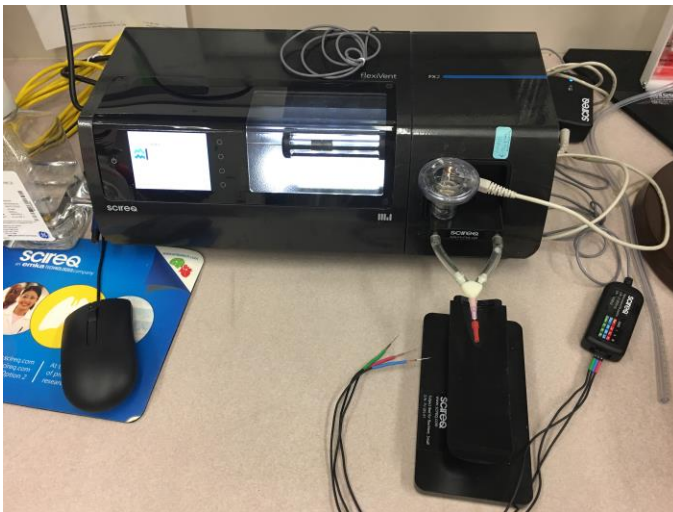
Adapted from Scireq's protocol for use in the Martin lab

Make up acepromazine dilution, methacholine doses, and decamethonium bromide solutions the morning of Flexivent (see recipes at the end of the protocol). Make sure that you contact Becca Martin to ensure she is able to make up ketamine/xylazine dilution and has you added as an authorized user prior to the start day.



### Day -1/0 Setup the Experiment

1. Turn on computer.
2. Ensure Flexivent setup is ready for use (see **Figure 1**).
3. The following items need to be check:



**Figure 1**

- a. Drying stones in tube – must be mostly blue! They do not dry if purple. Refill if necessary.
  - b. Y-tubing – needs to be dry and clean
  - c. Cannulas need to be clear and free of debris
  - d. Cannula caps should be damage free and able to seal on cannulas
4. Open the Flexivent program. Click on “Study Definition and Planning” and open “New Study”. Mice and groups need to be entered

5. Enter a Title, and Owner.
6. Click Next, and Under Subject Groups add the title of each group and the number of mice in each group.
7. Click Next. At the Subject Management section, click on the subject management button. To enter in each mouse (subject), click the mouse icon with the plus sign on it. You must give the mouse a unique name (I usually use my initials, the date, the group, and the mouse number ex. RKM HDM 615 6-12-19). A weight is required, but will be adjusted at time of testing, so as a placeholder put 20g. You will click to assign the mouse a group. If you do not assign the mouse a group, it will automatically assign the mouse to the first group and you can adjust this in the subject management window.

8. Don't forget to add an additional mouse called test, with the date. It will be used to calibrate the machine.
9. After all mice have been entered and checked for assignment to appropriate groups, exit.

### **Day -1/0 Calibrate the Machine**

10. Open Flexiware 8 program from desktop. Click on "Experimentation Session" and open.
11. Turn on Flexivent with power button on front of machine.
12. Upon opening, click on "Experiment Study"  
Flexivent Fx-mouse AN-VS (2) created by Becca. It is near the very bottom of the list. Click.
13. Make sure all elements are connected. Then verify module. Click "OK".
14. Select your subject called "test". Enter in most current weight (20g because you aren't actually calibrating with a mouse on the machine).
15. Wait for Flexivent Autocalibration (warm up time).
16. Remove Aeroneb from setup and add PBS. Click to Prime Aeroneb, vapor will come out the bottom. Put back on the setup.
17. Step 1 – channel calibration (Done once/experiment).
18. Cylinder calibration. Ensure entire system is closed by connecting manometer to the y-tubing and closing the syringe port to closed-after drawing up a full syringe of air.
  - a. Adjust pressure to 0 cmH<sub>2</sub>O (it should already be close). Click Next.
  - b. Push the syringe to 300 cmH<sub>2</sub>O. Watch bar go up (and keep there) and click next (square to the right will be green).
  - c. A set of max, min, and range values will pop up. The max and min absolute values should be similar and close to -80 and 80 cmH<sub>2</sub>O. It should only vary by maximum + or - 5cmH<sub>2</sub>O.
  - d. Repeat whole thing again.
19. Characterize nebulizer (done once per 14 days)
  - a. Weigh nebulizer with PBS/plug and drying tube with end yellow pieces attached, enter values. (Use anatomical scale)
  - b. Hook up drying tube to y-tubing and click "next".
  - c. Weight neb/drying tube (as in a.) and enter values.
20. Dynamic tube calibration (Done between each mouse).
  - a. Put cannula on and when prompted, put cannula cap on. This is the closed calibration.
  - b. Then with cap removed, click "next" for open calibration.
    - i. Want deep inflation  $R_i > 1125$ , snapshot  $R_t \leq 0.5$
    - ii. If these are off, redo the calibration (make sure no liquid in the tubing.-mouse will not run on the machine if there are any errors here)
    - iii. If  $R_t$  is off, change cannula, if  $R_i$  is off make sure all tubing is clean.
21. Calibration is complete! Do not need to start mouse default ventilation.

## **Day 0-Flexivent**

22. Start calibration on first mouse. Click Edit, "Edit subject management".
23. Click on current test mouse and click the return arrow, so that it can be used again. Make sure that the drop down box says pending and not terminal.
24. Move the first actual mouse into working site with the right arrow and enter weight (actually weight the mouse).
25. If you click okay, you will be prompted to repeat the dynamic tube calibration. The cannula that you utilize for this calibration should be the cannula that is used on the mouse.
  - a. Ensure there is PBS in the nebulizer. Calibration will not be correct without this. If this is after a previous mouse, ensure that the wash procedure is followed between mice to remove methacholine.
  - b. This will be done for each mouse.
26. After cannulating the mouse, attach mouse to flexivent with the mouse on its side. (This procedure is described in the addendum)
27. Click on "start ventilation"
28. After administering the paralytic (i.p.), you will want to ensure that the animal is paralyzed before proceeding. To do this, you will click first on the deep inflation. (27). This will allow any lungs that are collapsed from a long cannulation to expand. Then you will run a PV-P (27). It will be very obvious that the animal is paralyzed as it approaches the end of this breath hold. If it is not, it will take a spontaneous breath-which is evident by loops.
29. Attach EKG to mouse. Pattern is noted on module. Needles go in skin, just underneath on feet and arms.
30. Once paralyzed, click on "mouse mechanics scan".
31. Look for the small text on the left side of screen, which will indicate that the script has run to completion.
32. Click "Mouse Inhaled Dose Response"
  - a. The first script will run
  - b. Prompt for next dose (start with PBS). You will denote this as 0. Each dose increases. When script prompts for another dose, click yes if you are not finished. This is your time to change the dose and add the new to the nebulizer (100µl per dose). Use gauze to remove liquid between doses.
  - c. Then a new prompt will come up that will ask what the new dose is. The only thing that you change is the dose.
33. After the last dose is done, do not click for another dose. Then remove mouse and click the end ventilation button at the top of the screen on the left. It is the red button.
34. To move onto next mouse, open "Edit subject management". Click on the mouse in site 1 (the mouse that was just on the machine) and select "Valid" (keeps the data, most likely what you want) or "Invalid" (if something went so wrong that you don't want the data) and if the mouse is totally done, check the box that says that this mouse was "terminal". Now you can move the next

mouse into “site 1” and add a weight and proceed with the next dynamic tube calibration.

### Cleaning Between mice

35. Remove module, Y-tubing, nebulizer and cannula. Rinse nebulizer with wash solution (see recipe), then water. Do not get water in the electronics part where the nebulizer connects to the instrument. Rinse module only through the port where the mouse was attached. (See **Figure 2**) Be careful to avoid water in all other places. Use squeeze bottle to rinse with wash solution and



**Figure 2**

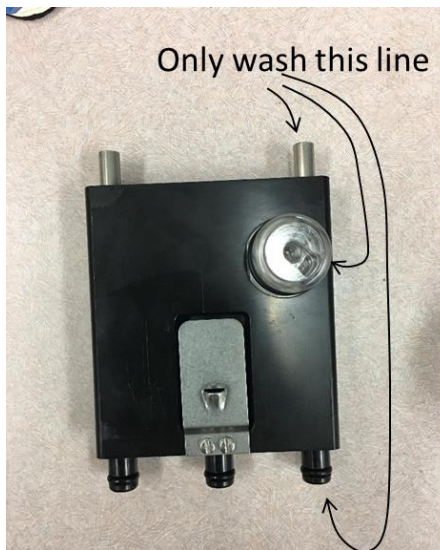
then water. Dry completely with canned air. Repeat this with cannula and Y-tubing.

### End Cleaning

36. First you remove the module completely. You insert the tubing into the sticker marked far right port on the front and the second tube into the sticker marked port on the side. When you click to exit the Flexiware, it

will prompt you to clean the machine. Putting the tubes in the wrong holes will ruin the machine! If you don't know-ask. (See **Figure 3**)

37. The first prompt will give you 5 minutes to push first Two 60mL syringes of wash solution. The second tube on the side should be put into an empty beaker. The front tube should be



**Figure 3**

be connected to the 60mL syringe. Once you click start-you should hear the valve open and you should begin pushing the liquid. If you do not hear the valve-or there is resistance-STOP! After pushing soapy water, push two-3 syringes of DiH2O through (pull back and forth a bit to clean the insides). Then push lots of air through to clear the water. Do this all in 5 minutes. Remove the tubes when done. The one on the side is a slide lock, do not pull, or it will break the machine.

38. You will then be prompted to put on the closed loop module. Then click start. This will dry the machine for 10

minutes. Once done the Flexiware will shut off. Remove the module and put in storage boxes in the drawer.

### **Recipes:**

#### Wash solution

1 Liter DiH<sub>2</sub>O

1 drop Dawn Gentle dish soap

#### Ketamine/Xylazine

Ketamine/xylazine working solution gives 100-150mg ket/10-15mg xyl per kg.

#### Acepromazine

Add 100uL stock to 900uL sterile saline. Each animal gets 60uL of this diluted solution. This needs to be made up each day and can be disposed of each day.

#### Decamethonium bromide

Decamethonium (0.5mg/mouse) – 0.01g in 2ml PBS, use 100µL per mouse

#### Methacholine

make a 100mg/ml solution (1g in 10ml or 0.5g in 5ml PBS) and do serial dilutions to get doses. Typical experiments use the following doses: 2.5, 5, 10, 25, 50, and 100 mg/mL

### **Addendum**

1. To insert the cannula into the mouse, first weigh the mouse and administer 150uL ketamine/xylazine solution i.p.
2. Inject 60uL Acepromazine i.p.
3. Wait 5-10 mins and move animal to heating area.
4. Administer second dose of ketamine/xylazine solution i.p. 100uL
5. For example, a 20g mouse should get about 250uL ketamine/xylazine.
6. During this procedure, you should toe pinch every 5-10 mins. When the animal is toe pinch negative, you may move forward to cannulation.
7. Rub small amount of Vaseline over the throat to keep fur back.
8. Cut as in diagram (Figure 4).
9. Pull Skin back and gently tease apart the glandular lobes.
10. Cut 5 inches of surgical sutures and utilize the pointed inverse tweezers to go behind the trachea and pierce the membrane. Once on the other side, place the thread in the tweezers and pull through. Leave thread behind.
11. Cut membrane open that surrounds trachea, until you see the white cartilage rings of the trachea. Cut a small slit in the trachea and insert the cannula, being careful to not cut completely thru the trachea.
12. Tie the suture tightly to secure the cannula in the trachea.
13. Place the animal on the ventilator.

