

DSI Ponemah

Rodent Head-Out Application Manual



OVERVIEW

This manual highlights how DSI's Rodent Head-Out Plethysmograph chambers interact with the Ponemah hardware and software as a complete system. This document will provide an overview of the chamber components, hardware connections, software calibration, and data acquisition.

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WELCOME

Congratulations on joining the community of users worldwide who rely on DSI's products to perform preclinical physiologic research. Thank you for your interest in DSI products. We are committed to providing you with quality products and services.

This manual will help you get to know your Ponemah Head-Out system for rodents. The structure of the manual was designed to sequentially guide you through using your DSI system from set up to data acquisition.

WHAT YOU WILL BE LEARNING

1. Understand how respiratory data is collected using Head-Out plethysmography.
 - a. Hardware
 - b. Software
2. How to setup your Head-Out chamber and hardware.
3. How to use the Ponemah software to calibrate the hardware and collect data

SCIENTIFIC BACKGROUND

Since the pulmonary system is considered a vital organ, regulatory agencies require that respiratory parameters be evaluated for any new compound for unwanted adverse effects. The most common parameters to measure are the tidal volume and breaths-per-minute.

ICH S7A document refers to the following for testing the respiratory system:

2.7.3 Respiratory System

Effects of the test substance on the respiratory system should be assessed appropriately. Respiratory rate and other measures of respiratory function (e.g., tidal volume (6) or hemoglobin oxygen saturation) should be evaluated.

Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies.

APPLICATION OVERVIEW

Head-Out plethysmographs are used for the study of restrained, conscious subjects. These chambers provide direct measurement of pulmonary flow signals with no temperature or humidity dependence, and no bias flow requirement. Chambers are available for a wide variety of species including; Mouse pup, Mouse, Rat, Hartley Guinea Pig, Rabbit, and NHP. This document will focus on Rodent. The Head-Out plethysmography system is ideal for researchers interested longitudinal studies without the use of anesthesia. The Rodent chambers feature the patented Allay™ restraint. Chambers are also compatible with other data acquisition systems with the addition of a compatible flow transducer.

Key Features:

- Continuously measures respiratory flow signals in conscious animal
- Chambers utilize easy to operate restraint systems and integrated pneumotach

Key Benefits:

- Patented Allay™ Restraint - secures the animal without compressing the thorax
- Species flexibility – software, amplifier and transducers support all Head-Out chamber designs
- Ponemah Software – integrated telemetry capabilities and flexible analysis
- Solid State Flow Transducer – small, rugged, low drift, high fidelity transducer mounts directly to chamber

Alternatives to the head-out method:

- A rodent whole-body chamber for flow and volume measurements that allows the animal to be unrestrained and freely moving.
- Large animal jacketed external telemetry (JET) with respiratory inductive plethysmography (RIP).
- Masked canine with pneumotachograph.
- NHP Helmet with pneumotachograph.

SYSTEM COMPONENTS

Depending on what you purchased, your system may have some or all of the following components:



1 – DSI ACQ7700

ACQ7700 6 slot signal conditioner chassis. With all slots filled with the Universal XE signal conditioners a maximum of 24 head-out chambers can be measure at a time.

2 – Universal XE Signal Conditioner

4 Channel Universal XE Signal Conditioner for use with the Flow transducers. Each Flow input uses a separate channel.

3 – Head-Out Plethysmograph

Plethysmographs are designed to accommodate one subject each. The subject is held via patented Allay™ Restraint, which secures the animal without compressing the thorax, and is then placed in the plethysmograph.

4 – Bias Flow Generator

This component provides a source of constant flow for calibration of the chambers. The DSI Buxco© Bias Flow Generator provides a 1 and 2.5 L/min flow rate in both Push and Pull directions. An alternative source of airflow may be used for calibration purposes, as long as it is constant and stable.

5 – Bias Flow Tubing and Filter assembly

The filter end fits into the bias flow generator and protects the pumps from debris within the chamber, while the opposite end connects to the chamber.

6 – TSI Flowmeter

The TSI Flowmeter is placed in line between the Bias Flow generator and chamber to provide an accurate measurement of the flow, which will be used as the High Cal value during calibration within the Ponemah software. This includes a variety of tubing and fitting sizes to fit your needs.

7 - Buxco Flow Transducer

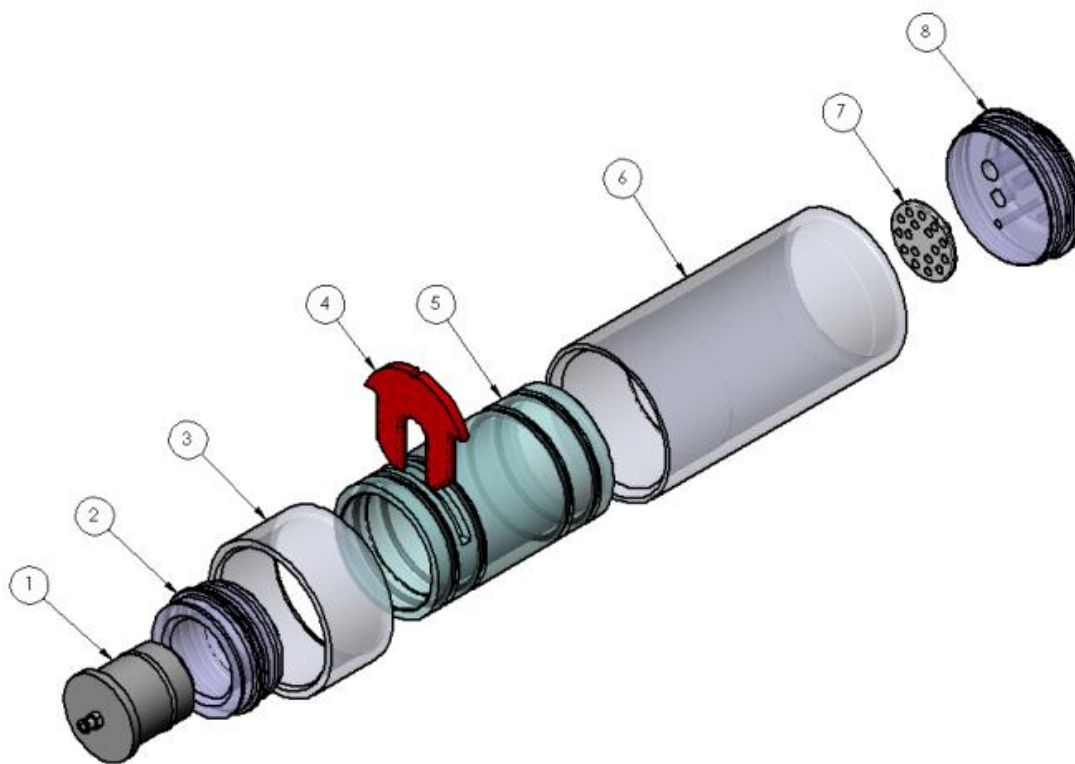
The flow transducer measures the pressure inside the plethysmograph. The pressure in the plethysmograph is linearly related to the flow of air moving in and out of the plethysmograph.

UNDERSTANDING THE RAT HEAD-OUT CHAMBER

The Head-Out Plethysmography chamber comes in three different sizes to fit the three approximate weight ranges:

Chamber	Approximate Weight Range (grams)
601-3921-001 (Small)	125-200
601-3921-002 (Medium)	200-400
601-3921-003 (Large)	400-600

Each chamber size contains multiple components to collect respiratory endpoints from restrained subjects. In this section, the form and function of the major chamber components are outlined.



1 – Calibration Plug

This is used to seal the chamber during the calibration process, as well as provide an inlet for the constant flow source to be used as the High cal.

2 – Neck Seal (Polycarbonate)

Removable silicone seal that fits around the neck to create an enclosed environment for the animal's thorax, thereby allowing the flow transducer to measure the respiratory parameters. One neck seal diameter exists for each chamber size, and each chamber comes with two neck seals.

Neck Seal for Chamber Size	Neck Seal Diameter
601-3925-003 (Small chamber)	5/8"
601-3926-003 (Medium chamber)	3/4"
601-3927-003 (Large chamber)	13/16"

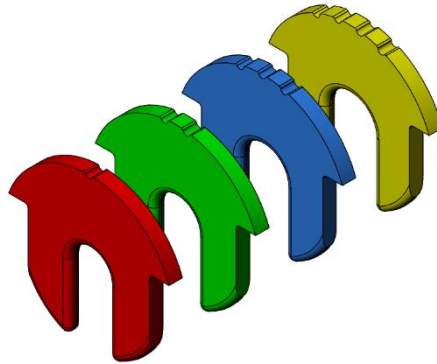
3 – Front Tube (Polycarbonate)

This piece slides overtop the Restraint Tube to hold down the Allay collar, thereby keeping the animal restrained without manual force being applied, as well as engaging with the front two O-rings on the Restraint Tube to create a tight seal.

4 – Allay Collar

The Allay Collar is a crucial piece of DSI's patented Allay restraint mechanism. Rather than traditional plunger techniques, which push the animal forward and lock their bodies in a compressed state that may affect the measured respiratory parameters, the Allay restraint uses a collar that fits around the animal's neck to hold it in place. This allows the animal to relax in a more natural state so that the thoracic cavity is not compressed.

Various sizes of collars are available with each size chamber: Red, Green, Blue, and Yellow, Orange, in order from smallest to largest. The size can be identified by both the color and the number of notches on the top arch of the collar. 1 notch being the smallest and 5 notches being the largest. Note: only the Medium size chamber has an Orange collar.



5 – Restraint Tube (Polycarbonate)

This is the tube where the animal will be restrained by the Allay collar. The four O-rings allow for easy connection and sealing with the Front Tube and Rear Tube in order to make a complete plethysmography chamber.

6 – Rear Tube (Polycarbonate)

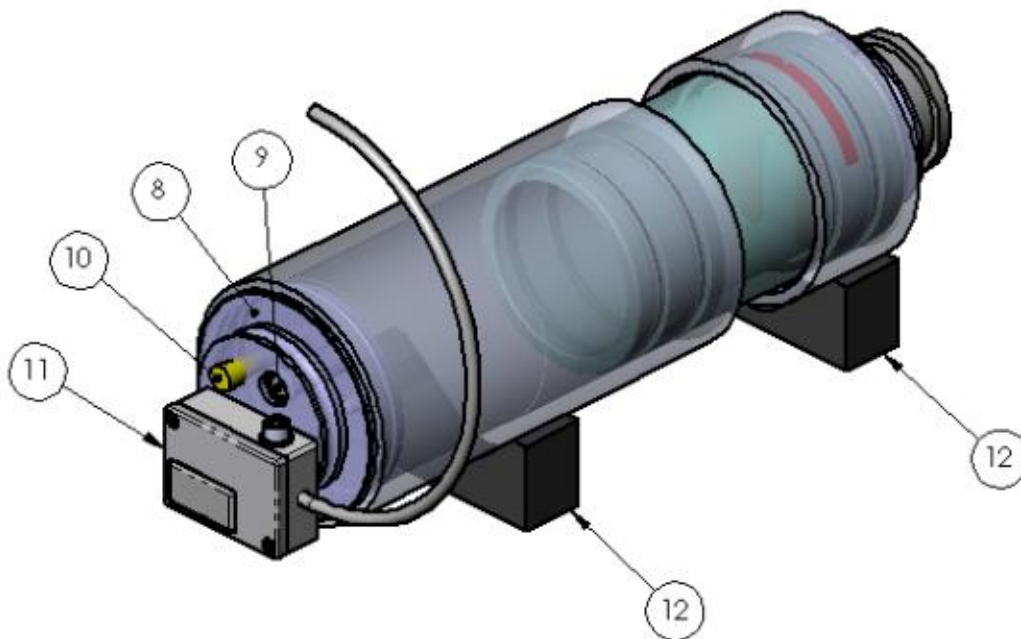
This is the tube that slides over the back of the Restraint Tube to enclose the animal's thorax. It may be pushed as far forward as necessary, but it must at least engage both O-rings on the back half of the Restraint Tube. The ability to adjust the rear tube depth allows the chamber volume to be optimized so the smallest required chamber volume can be obtained for a specific size rodent. Reducing the volume of the chamber will theoretically improve signal quality.

7 – Tail Guard

This plastic piece fits into the Plethysmograph cap and prevents the animal's tail from making contact with the pneumotach screen or transducer ports.

8 – Plethysmograph Cap (Molded Silicon)

This silicone piece seals the back of the Rear Tube and contains the pneumotach screens, access port for various hardwired options if desired, and flow transducer connection.



9 – Pneumotach Screen

The pneumotach screen is what allows the transducer to measure the varying pressure fluctuations in the chamber to calculate flow. This screen will get dirty over time from animal debris, dander, etc., thereby increasing the screen resistance and lowering the effective measurement range of the chamber. When this happens, the pneumotach screen can be easily removed for cleaning or replaced by popping it out of the Plethysmograph cap. See the Maintenance section of this manual for more information.

10 – Access Port (hole and plug)

This port is an opening through which accessory connection can be made such as hardwired connections or fluid lines can be made, if the application requires. This is not used for normal chamber operation. Note: a new Plethysmography Cap will have a silicone membrane covering the Access Port hole and therefore will not need to be plugged if not using the Access port. To use the access port simply cut the membrane away with a small knife. After removing the membrane, the hole will need to be sealed “airtight” with either with a fitting allowing the wires or fluid lines to pass through it, or the supplied yellow plug.

11 – Flow Transducer

The Flow Transducer connects to the ports on the Plethysmograph Cap. Ensure that the (+) side is connected to the port leading to the animal chamber and the (-) side is open to atmosphere. This component measures the pressure fluctuations within the chamber as the animal inhales and exhales, which the software then equates to flow and volume via the calibration procedure. See the Calibration section of this manual for more information.

12 – Chamber Cradle

These pieces allow the Head-Out Chamber to rest in a stable, upright position when placed upon a flat surface.

HARDWARE SETUP

Before setting up the apparatus, choose a location which has sufficient space for the equipment and plethysmograph chambers. One power receptacle is required for the ACQ7700 and another for the Bias Flow (if being used for calibration), as well as one for the TSI Flowmeter during chamber calibration. The ACQ7700 must be within reach (via USB Cable) of the PC to be used for data acquisition.

Hardware setup consists of the making the physical connections of the chamber and computer to the apparatus. In addition, the hardware must be configured within the software in order to calibrate the apparatus and perform data collections.

CONNECT THE CHAMBERS

For each plethysmograph, perform the following connections:

1. **Attach the Flow Transducers.**

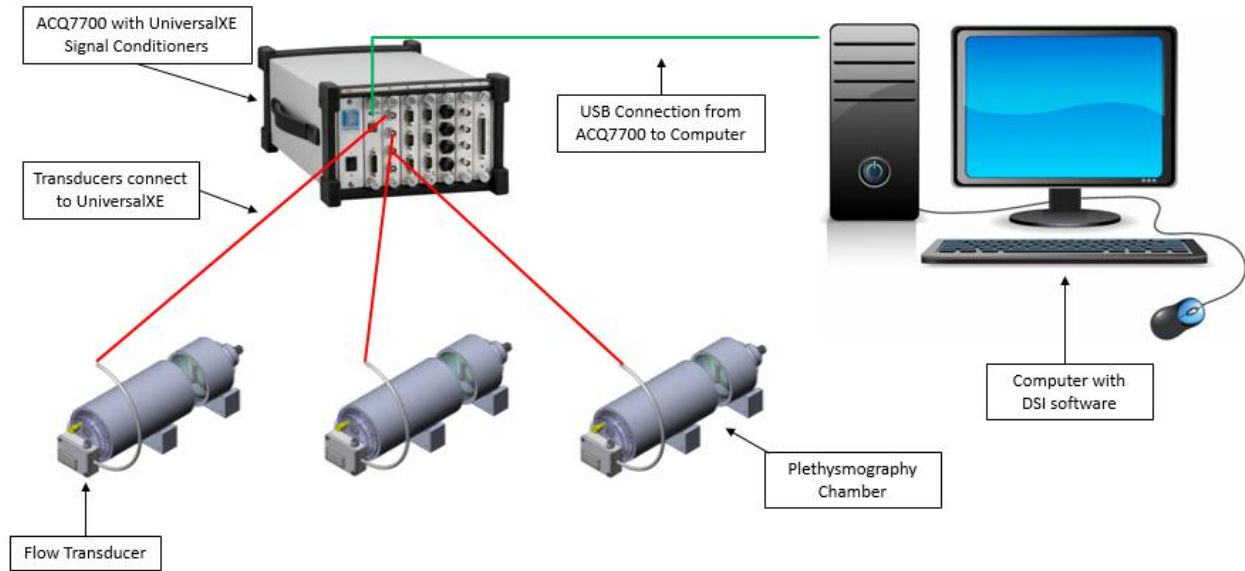
Place chambers on a stable surface free from vibrations, preferably a separate surface from the Bias Flow to prevent pump vibrations from appearing on the flow signal. Connect the flow transducer to the chamber. Orient the transducer so the “DSI” logo is right-side up.

2. **Connect the bias flow.**

Plug the filter end of the bias flow tubing into the Bias Flow generator. Connect the other end of the tubing to the Flowmeter. See Calibration section for additional information.

CONNECT THE HARDWARE

The following block diagram is a typical representation of the connections that are needed for the physical setup of the system for three Head-Out chambers.

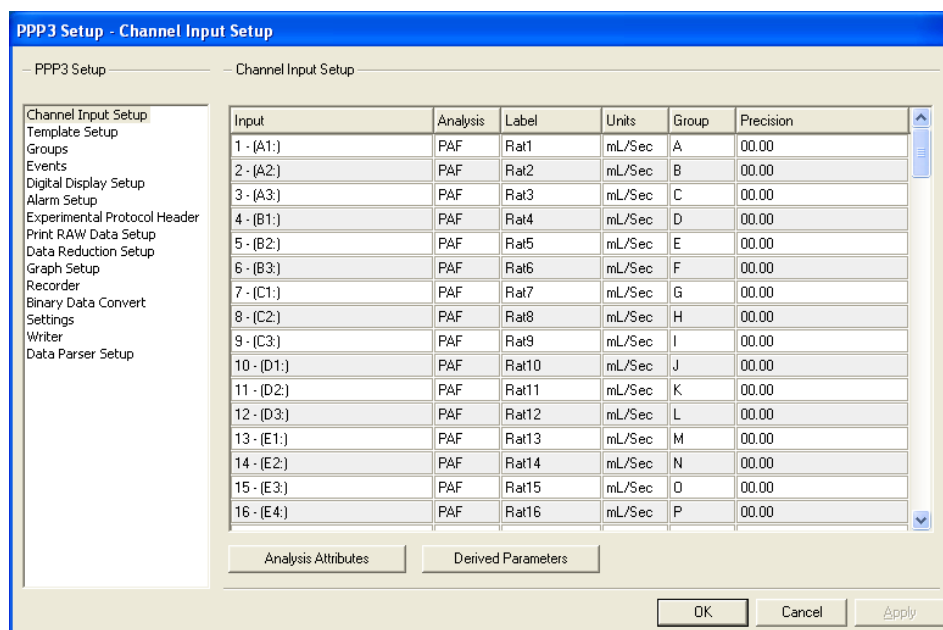


CREATING HEAD-OUT PROTOCOLS

This section explains the **P3 Setup**, from the **Setup** menu, for performing the desired respiratory measurements to calculate the **Head-Out** derived parameters.

CHANNEL INPUT SETUP

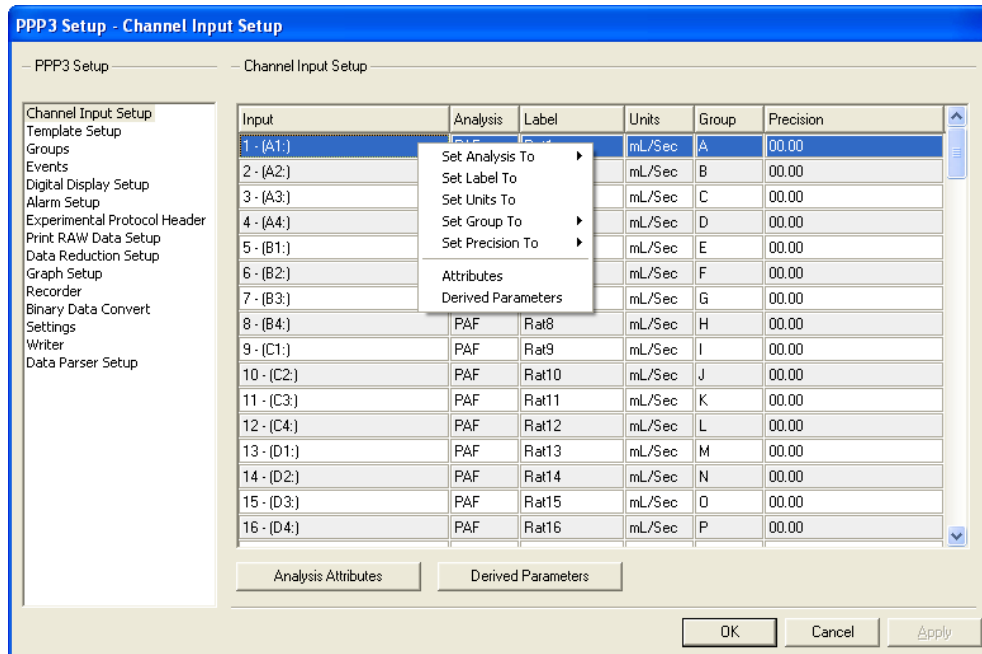
From the **Setup**, **P3 Setup** menu, select **Channel Input Setup** (shown below).



Channel Input Setup Configuration

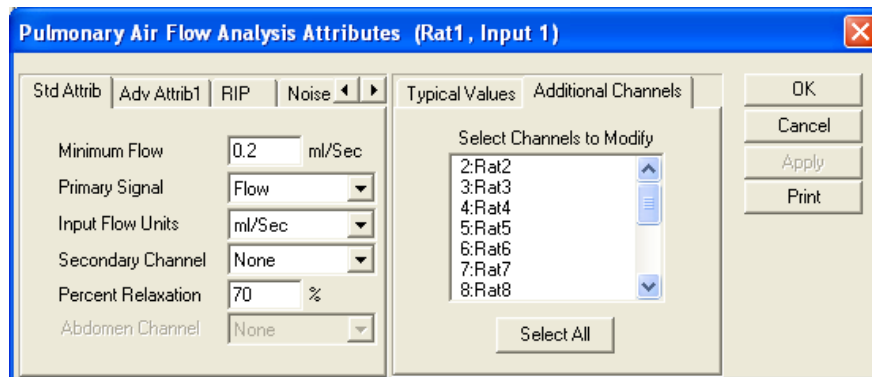
Note: The number of channels configured depends on the number of animals to be utilized in the experiment. A single channel of flow is needed to be allocated per test subject.

1. Set the Analysis Module for Flow channels to PAF (Pulmonary Airflow). All channels that will not be sampled should have their Analysis set to DIS (Disabled).
2. Set channels from the same subjects as the same Group.
3. Select **Derived Parameters** (shown below, P3 Setup Dialog) or if all channels are to have the same derived parameters, the user may hold the <Shift> or <Ctrl> key down to multiselect the channels and then right click to set the derived parameters. You will be presented with a list of derived parameters that may be selected by left clicking the mouse next to the output desired. This output will be presented in spreadsheet format during acquisition and subsequent Replay/Review. These parameters may be added or deleted during post processing provided the RAW data file is being saved.



P3 Setup Dialog

- Right click on Input one and select Attributes. Select the appropriate Input Flow Units. Typically, this is mL/Sec for rodents and L/Min for large animals. If this is not set appropriately then the calculated Tidal Volume and Minute Volume parameters will be incorrect.



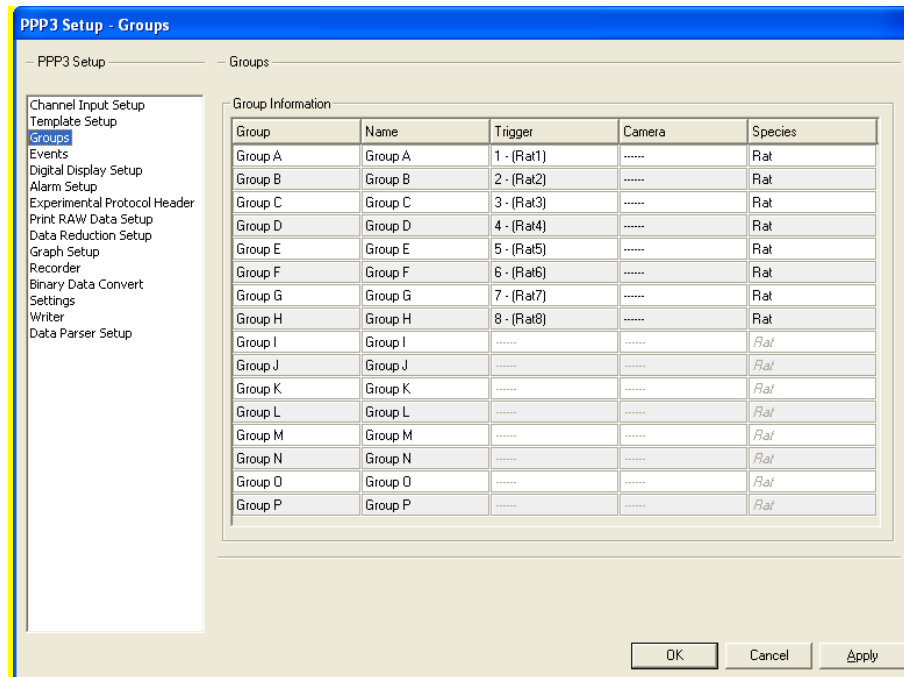
PAF Attributes Dialog

- If more than one channel needs to be setup or changed, the Additional Channels tab allows the selection of multiple channels that the attributes can be applied to.

GROUPS

In the **Groups** configuration, the user is able to assign a name to each group that was assigned in the **Channel Input Setup** menu and select the appropriate species. Choosing the correct species will allow Ponemah to populate default settings that are unique to the animal. If the species being used does not appear in the pull-down menu, select a species based on a similar heart rate.

When logging data in a beat-to-beat mode (Epoch) a Trigger channel must be set for each group. The Trigger channel will be used as the basis for determining when to update the derived data table. The trigger channel should be set to a reliable signal or to the phenomenon that occurs first. In this example, the trigger channels were set to the PAF channel for each animal.



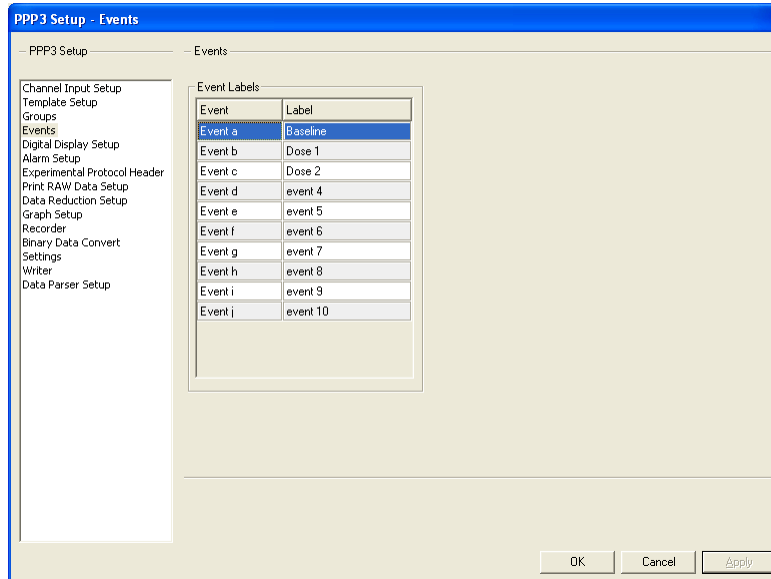
Groups Dialog

EVENTS

During the course of the acquisition, events can be entered that document the action taken, such as a baseline reading was started, or a dose was given.

If events are going to be used, the user can setup these events prior to data collection and enter the needed descriptions for the events.

From the **Setup, P3 Setup** menu, select **Events** (shown below).



Events Dialog

DIGITAL DISPLAY SETUP

From the **PPP3 Setup** dialog, select **Digital Display Setup**. Digital Display is often used to display real-time derived parameter values that are of specific interest to the user. The values will be automatically updated based on the **Logging Method** chosen (See **Systems Settings** below).

While in the **Digital Display Setup**, users can modify these channels individually or hold the **<Control>** key down and select multiple channels and then right click to set the desired values.

PPP3 Setup - Digital Display Setup

PPP3 Setup Digital Display Setup

Choose Font: Always on top

Channel Input Setup
 Template Setup
 Groups
 Events
Digital Display Setup
 Alarm Setup
 Experimental Protocol Header
 Print RAW Data Setup
 Data Reduction Setup
 Graph Setup
 Recorder
 Binary Data Convert
 Settings
 Writer
 Data Parser Setup

	Channel	Parameter (Start)	Label	Txt Clr	Bkg Clr
<input checked="" type="checkbox"/>	1 - (Rat1)	BPM	Rat 1 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	1 - (Rat1)	MV	Rat 1 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	4 - (Rat2)	BPM	Rat 2 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	4 - (Rat2)	MV	Rat 2 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	7 - (Rat3)	BPM	Rat 3 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	7 - (Rat3)	MV	Rat 3 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	10 - (Rat4)	BPM	Rat 4 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	10 - (Rat4)	MV	Rat 4 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	13 - (Rat5)	BPM	Rat 5 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	13 - (Rat5)	MV	Rat 5 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	16 - (Rat6)	BPM	Rat 6 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	16 - (Rat6)	MV	Rat 6 MV	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	19 - (Rat7)	BPM	Rat 7 BPM	<input type="text" value=""/>	<input type="text" value=""/>
<input checked="" type="checkbox"/>	19 - (Rat7)	MV	Rat 7 MV	<input type="text" value=""/>	<input type="text" value=""/>

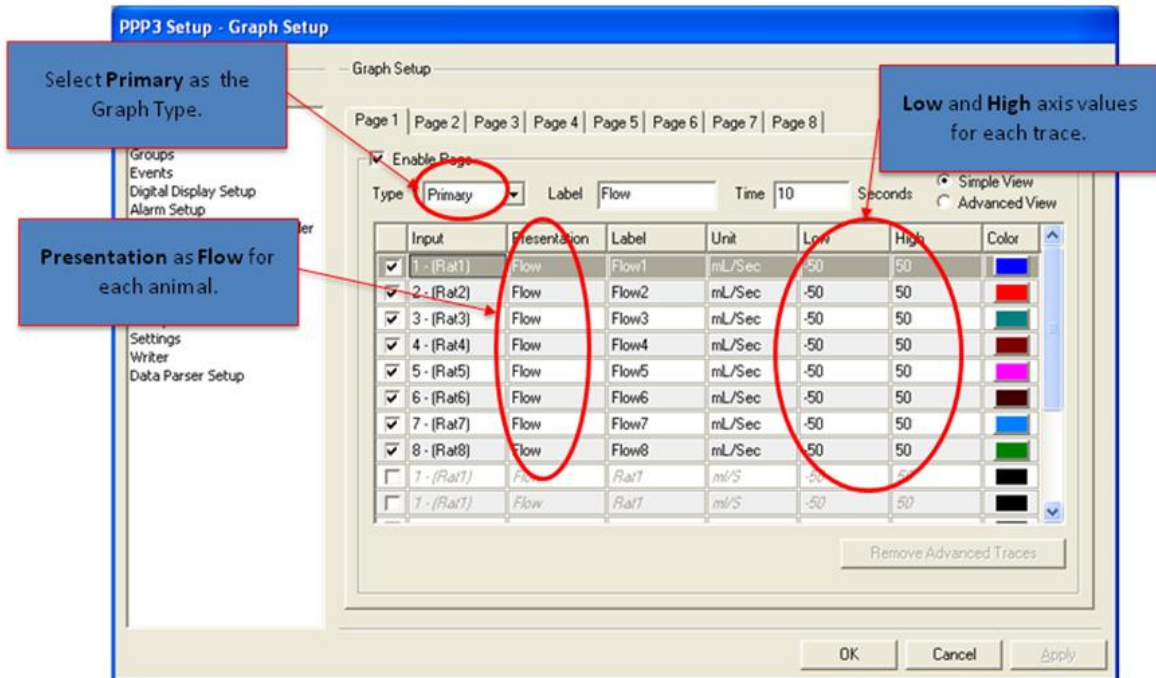
Timer Action on Completion

OK Cancel Apply

Digital Display Setup

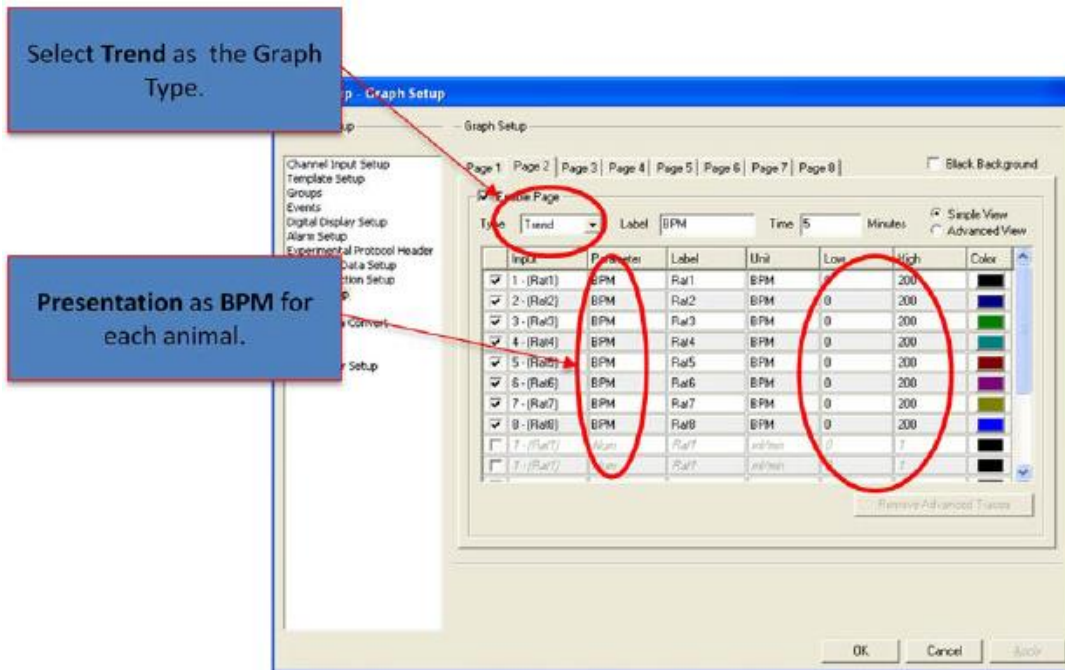
GRAPH PAGE SETUP

From the PPP3 Setup dialog (shown above), select **Graph Setup**. Choose **Primary** as the **Type**. Using the **Simple View** (default selection), configure the **Presentation Signal** so each animal has one axis displaying the flow signal and one axis displaying the volume signal. Next, set the low and high flow values for the Y-axis scaling. The X-axis, or sweep rate for the graph page, is set in the box labeled **Time** and for this application 10 seconds is a good starting point.



Primary Graph Page

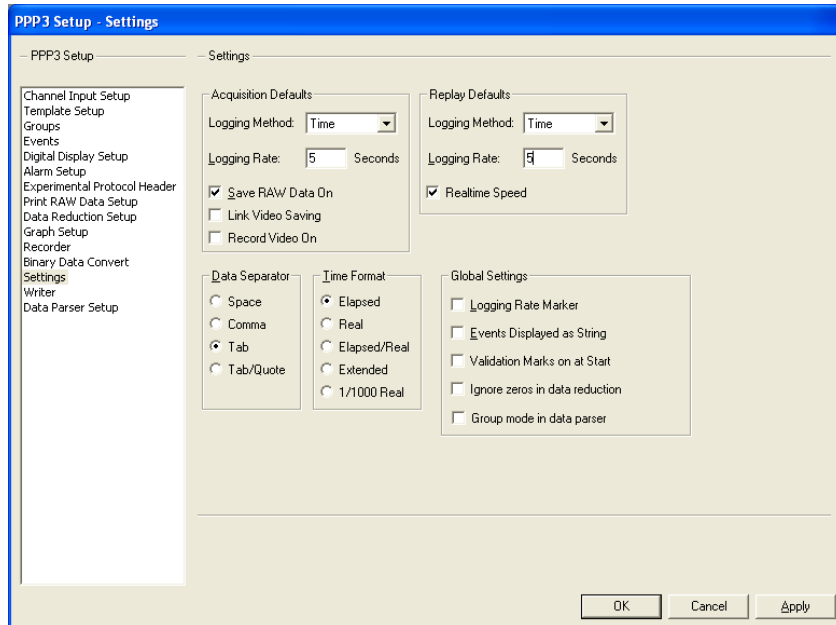
For displaying **Trend** data such as Breaths per Minute (or Tidal Volume), a **Trend Graph** can be setup by selecting **Trend** as the **Type**. The x-axis, or sweep rate for the graph page, is defined in the box labeled **Time**. For this particular application, 5 minutes is appropriate.



Trend Graph Page

SETTINGS

Select the **Settings** configuration. Under **Acquisition Defaults**, select the desired **Logging Method** and **Rate**. This will determine how often the data is averaged and logged. This rate can be changed at any time during acquisition or subsequent post processing of the data. Next, select **Save RAW Data On**. This will ensure that data is automatically saved to the hard drive and subsequent review and analysis will be allowed.

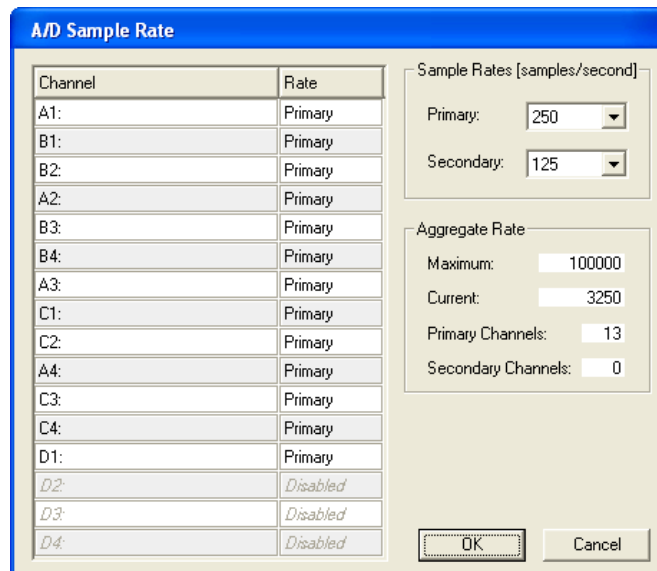


Settings Configuration

ACQUISITION SAMPLE RATE

From the **Acquisition** pull-down menu, select **A/D Sample Rate**. Set the sample rate to the appropriate Hz for the active channels in the Sample Rate column. Typical Sample Rates for this type of application is 250 Hz.

Finally, go to the **File** menu and select **Save As**. Provide a name for the protocol that was just configured. The initial setup is now completed. Consult the Ponemah manual for a comprehensive list of available features.



WBP Compensated - Sample Rate Dialog

NOTE: Cross channel calculations between the flow signal and the temperature/humidity signals require the sampling rate to be the same and set to Primary.

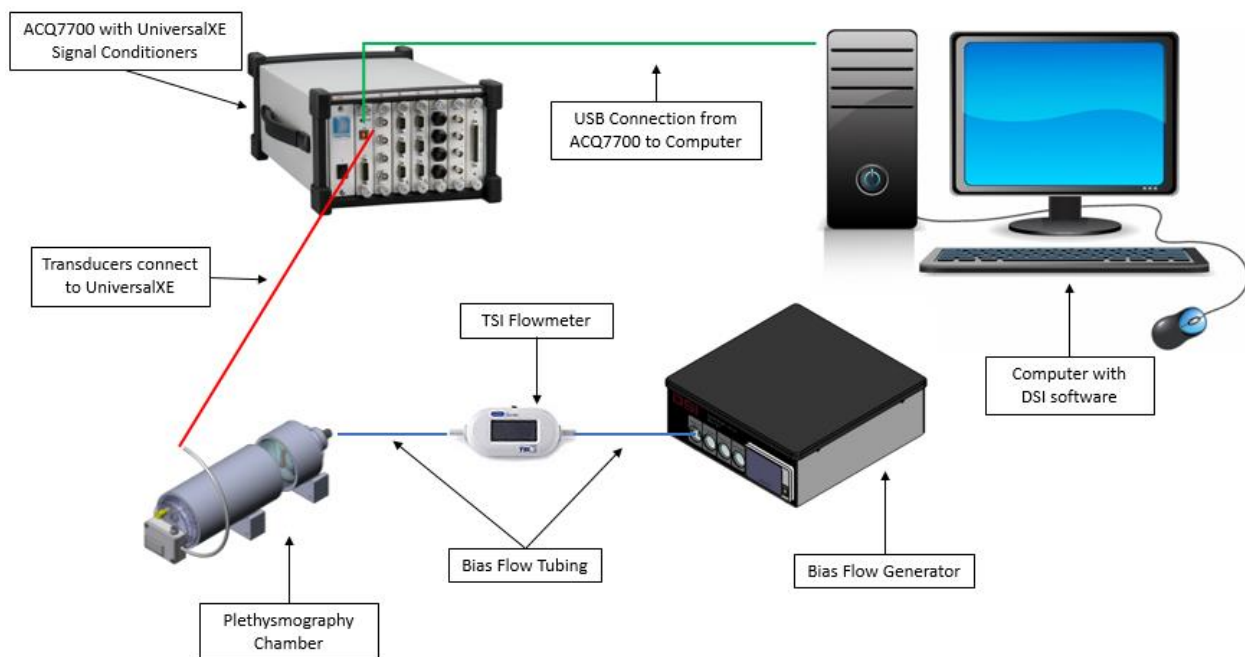
CALIBRATION

Note: It is recommended to calibrate your Head-Out sites once per day, prior to the commencement of your acquisitions for that day.

PREPARE

Before you initiate calibration, please ensure the following:

1. The plethysmograph is empty (no animal).
2. The plethysmograph is closed tightly and sealed with the Calibration Plug.
3. Ensure the Access Port in the Plethysmography Cap is sealed with either the original membrane, yellow plug, or a fitting allowing wires or fluid lines to pass into the chamber.
4. Perform all connections as described in the **Connect the** section of this manual.
5. The TSI Flowmeter is connected in line between the Bias Flow generator or regulated lab air source and the luer fitting on the Calibration Plug.
6. An applicable Protocol file has been created in the Ponemah software.



FLOW/VOLUME CALIBRATION

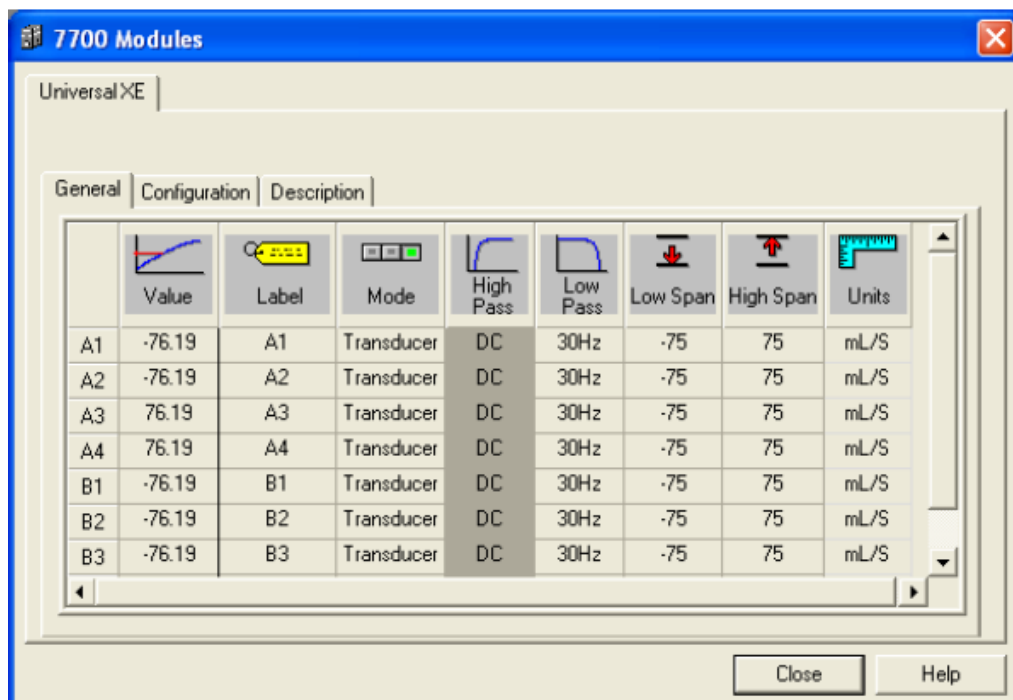
There is a two-step calibration procedure for the chamber flow signal. First take a measurement of the zero flow, then apply a constant flow and take another measurement.

In order to calibrate the chamber, all ports must be closed off so that the only air flow is through the pneumotach. The only other connection to the chamber is the constant flow.

NOTE: For the correct Volume calculation, the inspiration portion of the signal must go in the positive direction. If the inspiration has a negative deflection the user can use the Invert Flow attribute in the Analysis Attributes for the signal.

From Hardware -> 7700 Amplifier Setup, the ACQ7700 calibration menu will be displayed

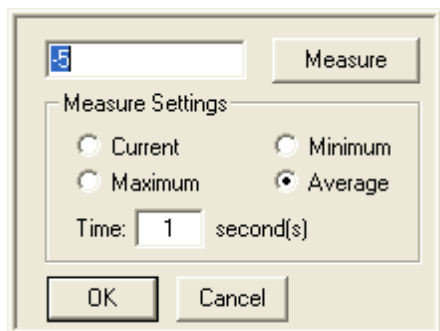
1. In the Universal XE | General tab:
 - a) Set the Mode to Transducer.
 - b) Set the Low Pass Filter to 30Hz.
 - c) Leave the other settings at default values.



UniversalXE General Tab

2. In the Universal XE | Configuration tab:
 - a) Set the excitation to 5V.
 - b) Set the Units to mL/sec.

- c) Enter 0 as the Low Unit based on the actual flow values you will use for calibration.
- d) With no flow being provided to the chamber, left-click the Low Cal cell associated with the Pressure Transducer channel.
- e) Select the Measure button and wait for the Low Cal cell value to automatically populate.



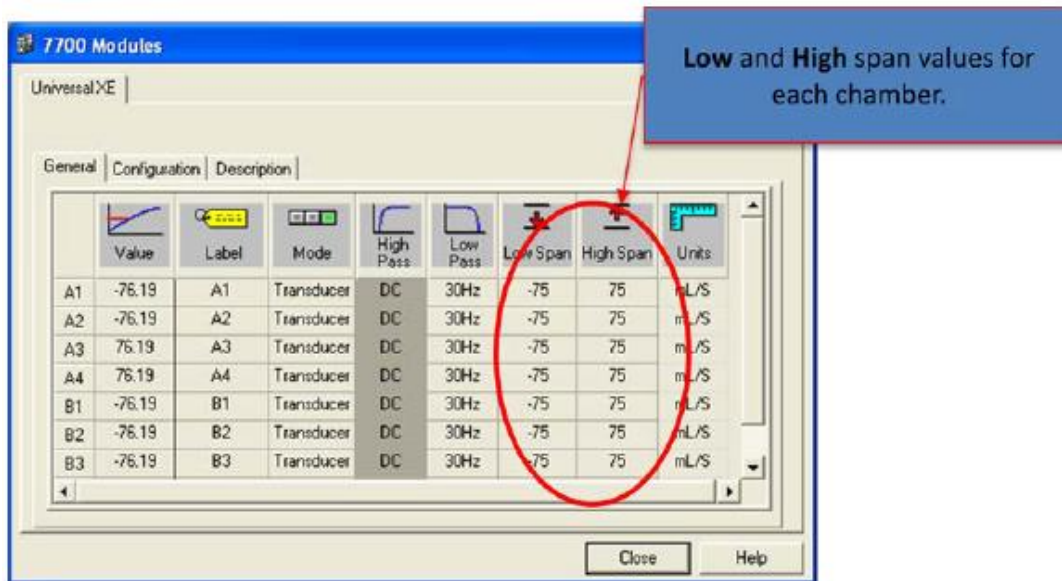
- f) Apply the bias flow to the chamber and wait for the flow in the chamber to stabilize. DSI supplies a TSI Flow Meter to be placed between the bias flow and chamber to obtain an accurate flow rate and know when the desired flow has been achieved.
- g) Convert the value shown on the TSI Flowmeter from L/min to mL/sec and then enter this value as the High Unit
- h) Left-click the High Cal cell associated with the Pressure Transducer channel.
- i) Select the Measure button.
- j) The Value cell associated with the Pressure channel provides real-time, calibrated data from the transducer connected to the channel. It may be used to test the calibration by applying various flows through the chamber.

	Value	Excitation	Low Cal	High Cal	Low Unit	High Unit	Units
A1	-50.794	5V	-0.076	0.668	0	25	mL/S
A2	50.794	5V	-0.076	0.668	0	25	mL/S
A3	-50.794	5V	-0.076	0.668	0	25	mL/S
A4	-50.794	5V	-0.076	0.668	0	25	mL/S
B1	-50.794	5V	-0.076	0.668	0	25	mL/S
B2	-50.794	5V	-0.076	0.668	0	25	mL/S
B3	50.794	5V	-0.076	0.668	0	25	mL/S

UniversalXE Configuration Tab

This procedure must be performed for each chamber's Flow channel.

3. Once all the chambers have been calibrated, the **Low Span** and **High Span** in the **General** tab must be checked that they are the correct settings (see **Typical Species Values** table in the **Appendix** for examples).



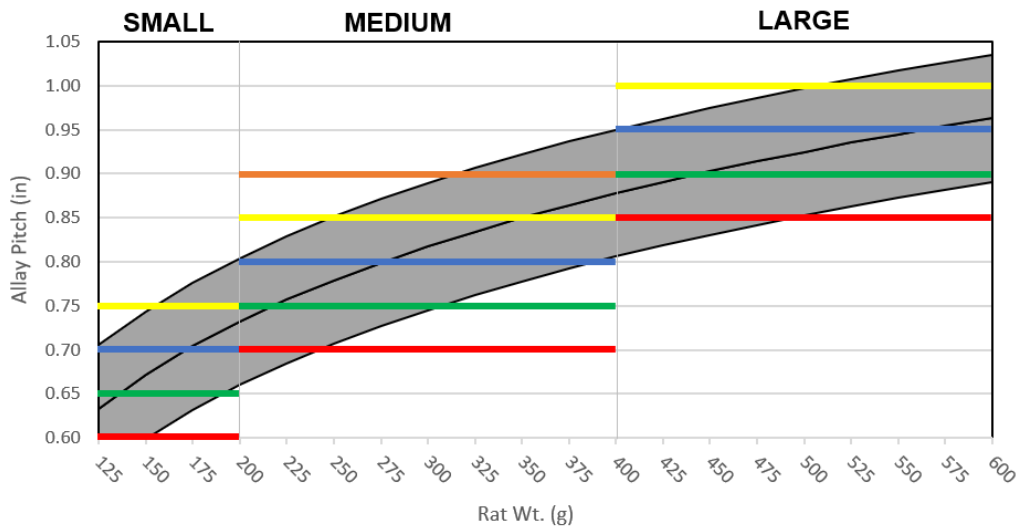
LOADING THE ANIMAL

This section depicts the various steps required to properly position and load the animal into an Allay Restraint chamber. Practice and confidence will allow a single investigator to easily perform this procedure.

CONSIDERATIONS

- The rodents that will be used should be properly acclimated to the chambers restraint system over a period of a few days prior to the collection of data.
- The hardware and software systems are ready for operation prior to placing the animal in the Allay restraint chamber
- The investigator should start with the largest restraint collar and work downward to identify the most appropriate size. The Allay Collar sizing table below will assist the user in selected the most appropriate size to start with for a specific size subject. Once comfortable with the process and the typical size of the animals used, choosing the appropriate collar will become easier. It is recommended that collar selection is done prior to inserting the rodent into the restraint tube.

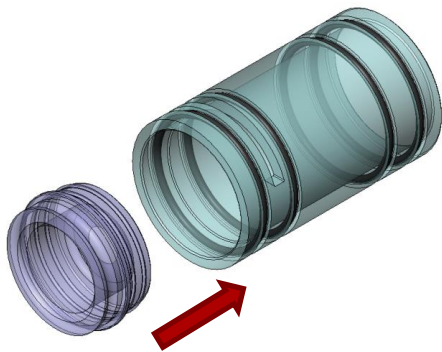
Sprague Dawley Rat Weight Vs. Alloy Pitch: $\pm 1 \sigma$



- O-rings can be lubricated using silicone grease to make it easier to insert/remove components. Place a dab on your fingertip and run your finger around the entirety of each O-ring and interior of the Front Tube and Rear Tube to ensure smooth assembly. The silicon grease supplied is a food grade silicon grease.

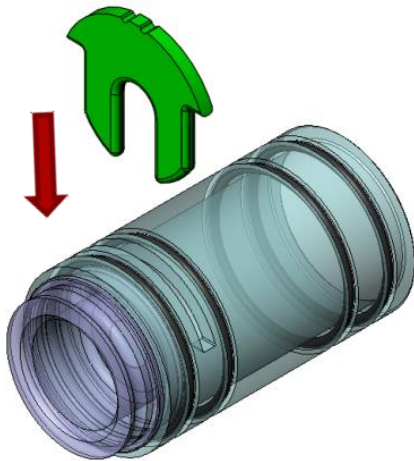
SUGGESTED ANIMAL HANDLING PROCEDURE

1. Insert the Neck Seal into the end of the Restraint Tube that has the Alloy Clip slot.

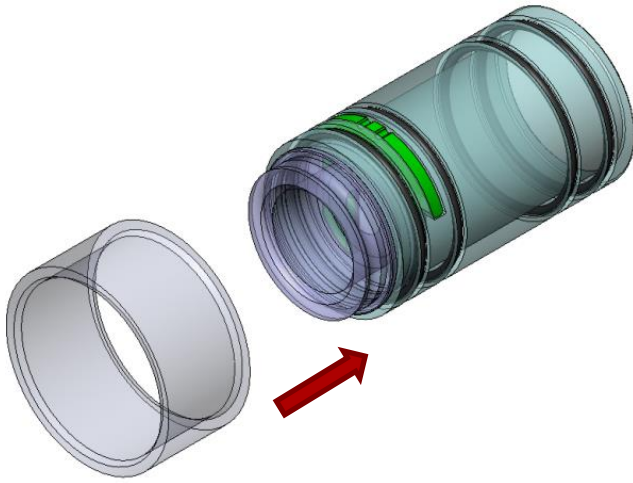


2. Pick up the rat by the scruff or the base of the tail.
3. Load the rat into the rear of the Restraint Tube and gentle push it toward the Neck Seal by the base of the tail.
4. Once it reaches the Neck Seal apply more tension to the tail to hold the rat in place.

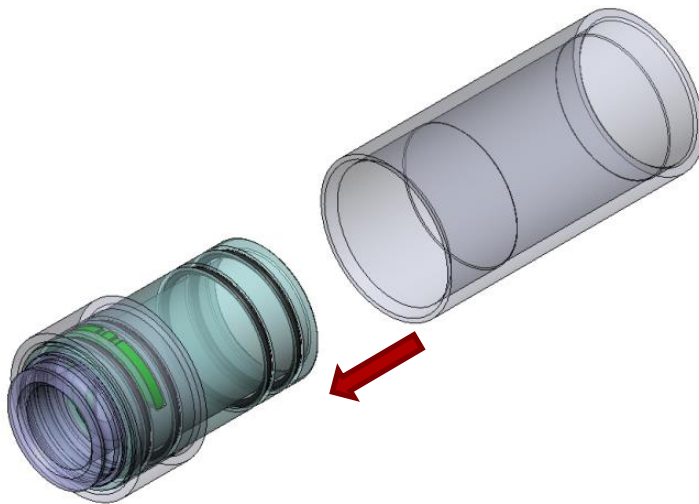
5. Use your judgment to decide when the rat is in a good position to gently push the rat's head through the seal. Typically, the rat's curiosity will lead it to explore the opening in the seal. Using a treat will also help promote the rat to push its head through the seal. Guide the rat's head through the seal until the ears have passed through the seal opening. Be careful to not let the rat try to proceed further through the neck seal as it could tear the seal with its nails.
6. Holding the rat and restraint in one hand, use your other hand to pick up the appropriate size of the restraint collar.
7. While continuing to securely hold the rat by the base of its tail, carefully insert the restraint collar through the slot in the restraint tube aligning it over the animal's neck. The Allay Collar should be positioned behind the ears, and in front of the shoulder blades. Moving the rat slightly forward and backward by the base of its tail while gently pushing down on the Allay collar will often help with insertion.
 - a) If the animal is able to back out, the collar is too big. Proceed to the next smaller size collar
 - b) NOTE: Animals will inherently resist the process. Always observe the animal's behavior for any signs of significant stress and respond accordingly. As mentioned in the Considerations section, chamber acclimation over a period of a few days is the best way to reduce the animal's stress, improve the researcher's animal handling experience, and improve data quality.



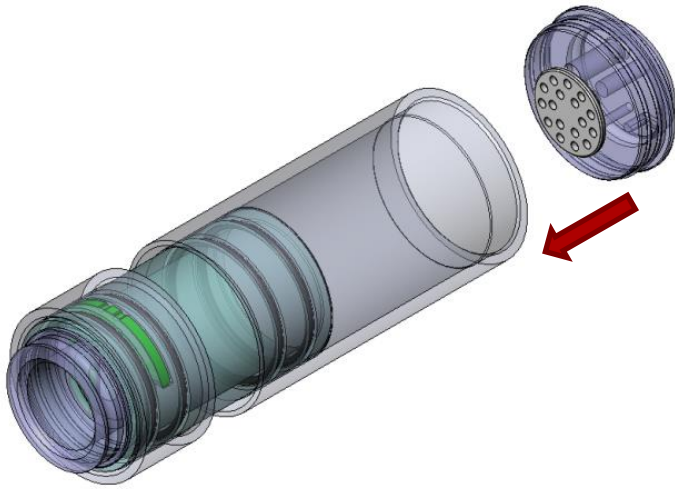
8. Once the appropriately sized Allay restraint collar has been properly fitted, use a finger to keep it in place and slide on the Front Tube piece so that it is over both of the front O-rings of the Restraint Tube.



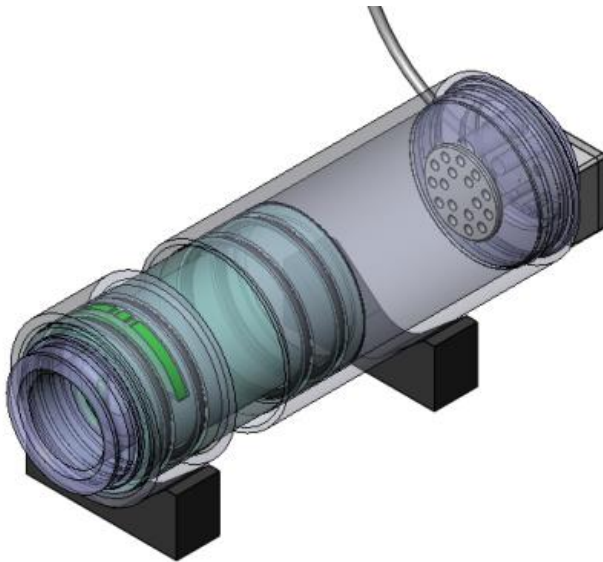
9. Now that the Neck Seal, Alloy Collar, and Front Tube are in place, confirm that the Neck Seal is properly seated around the rat's neck with the ears and whiskers completely on front side of the seal. This should ensure an airtight seal around the rat's neck.
10. Next carefully slide the Rear tube over the Restraint tube making sure not to pinch the Rats feet or tail. Ensure the Rear is inserted far enough that it covers both of the rear O-rings of the Restraint Tube. Alternatively, the Rear Tube can be slid onto the Restraint Tube before inserting the rat into the Restraint tube (Step 3).



11. Adjust the position of the Rear tube to fully encompass the rear legs of that Rat and leave ample room for rat's tail.
12. Push the Rear Cap onto the back of the Rear Tube piece with the screen at the 12 o'clock position.



13. Set the chamber into the chamber cradle
14. Connect the flow transducer to the Plethysmograph Cap.



SUGGESTIONS FROM THE RESEARCH COMMUNITY

This section provides additional tips and suggestions to allow the investigator to become more comfortable with the Allay restraining process. These suggestions have been compiled from a number of Allay restraint users. They have been provided for your reference.

Acclimation is suggested prior to starting a study:

- Investigators should consider acclimating animals to the restrainers prior to the start of a study. Acclimating the animal allows for reproducible animal behavior and consistent data

Animal sedation is not required when using the Allay restrainer

- Sedation may be useful for a new user trying the Allay restrainer for the first time
- Sedation should never be used while the animal is on a study

Location of loading an animal is important

- Do not hold the chamber in the air or over the end of a table or countertop. The animal will feel more comfortable if they can see a flat surface beneath them.

Removing the animal from the Allay restrainer

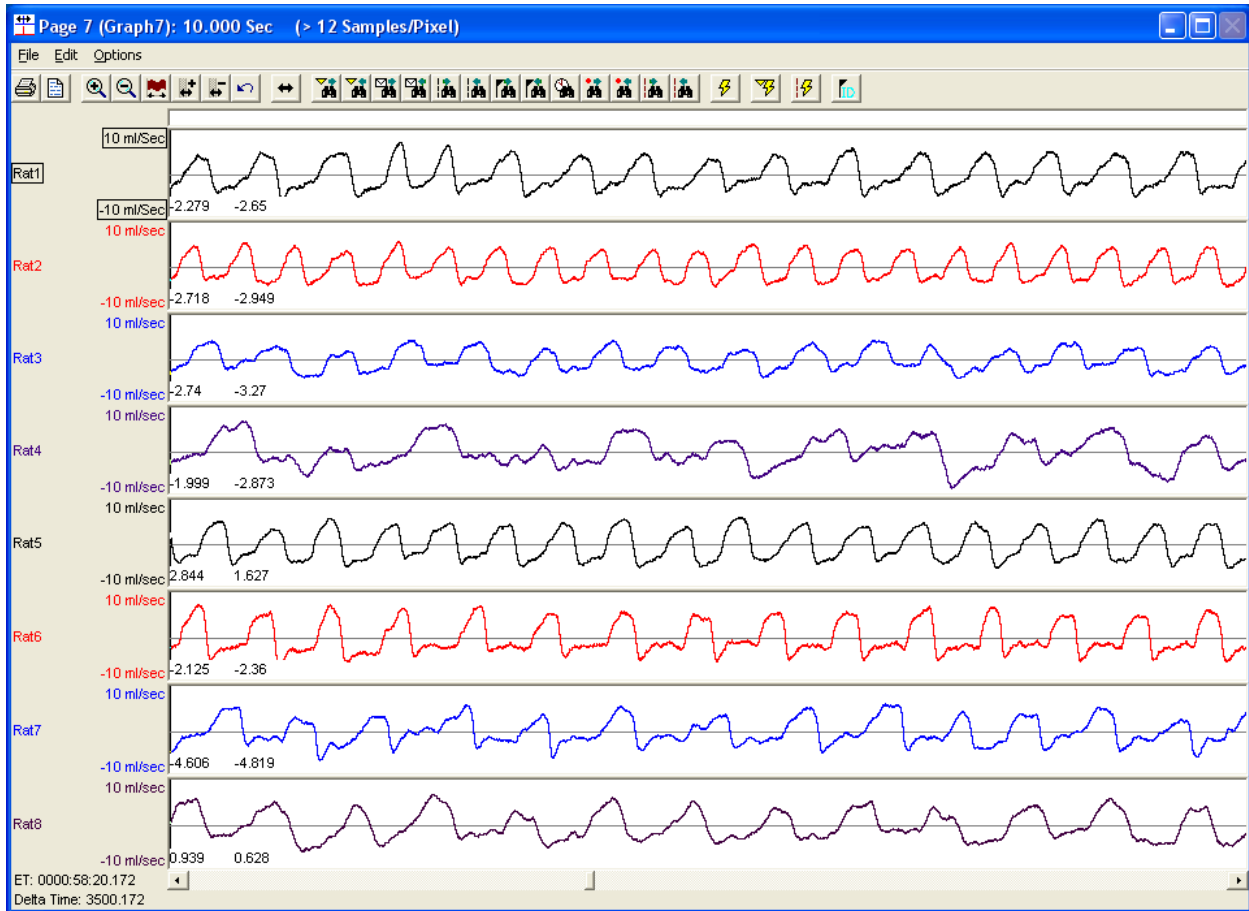
- It is recommended to first remove the Rear Tube and then remove the Allay collar to allow the animal to back out of the Restraint Tube.
- If the animal will not back out of the restrainer, carefully remove the neck seal.

STARTING DATA ACQUISITION

The application is now ready to collect and analyze data after the animals have been placed in the chambers.

From the **Acquisition menu**, select **Data Set Name** and enter a name that will be used to name the data files.

The user can then start the acquisition from the **Acquisition menu** by selecting **Start Acquisition**. This will show data being collected on the graph page which should be similar to the image displayed below.



From this point the user can:

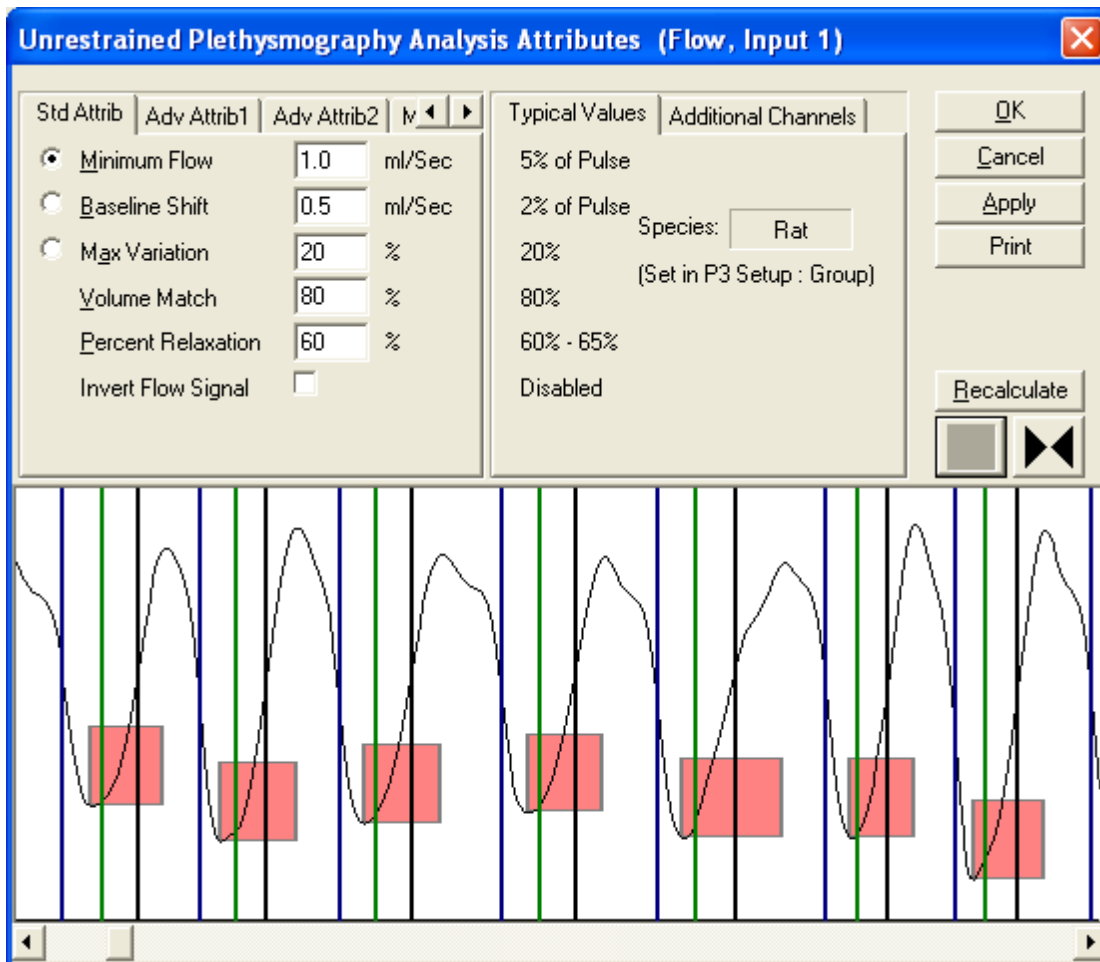
- Add events at certain points in time to signify certain activities such as an animal being dosed.
- Change the graphical appearance to modify items such as Y-axis scaling or the time base.
- Adjust data logging rates (time or epoch).
- Toggle the saving of the waveform data on and off.

For more information on all of these functions please see the related Ponemah manuals.

Once the desired amount of data has been collected and analyzed, the user can stop the acquisition through the **Functions** menu by selecting **Stop Acquisition**.

DATA REVIEW

The user can now review the data to make any adjustments needed. Please refer to the Review Manual (MU00196) for assistance.



MAINTENANCE

As you use your Head-Out system, some parts will wear out and may need to be replaced. Proper care and maintenance will help ensure consistent results and that your equipment investment will last for years.

Consider replacing/servicing the following components on a scheduled basis:

- Tubing
- Pneumotach Screens
- O-rings

The frequency of replacement will depend on a variety of factors that may include: testing environment, frequency of use, and proper care of equipment throughout. Good Laboratory Practices (GLP) and routine equipment maintenance/servicing are recommended. At a minimum, O-rings and screens should be replaced annually.

ACCESSORY TOOL KIT

The Basic Chamber Accessory Tool Kit provides the necessary tools required for routine maintenance.

- Replacement part number: 601-6600-001

The tool kit consists of the following items:

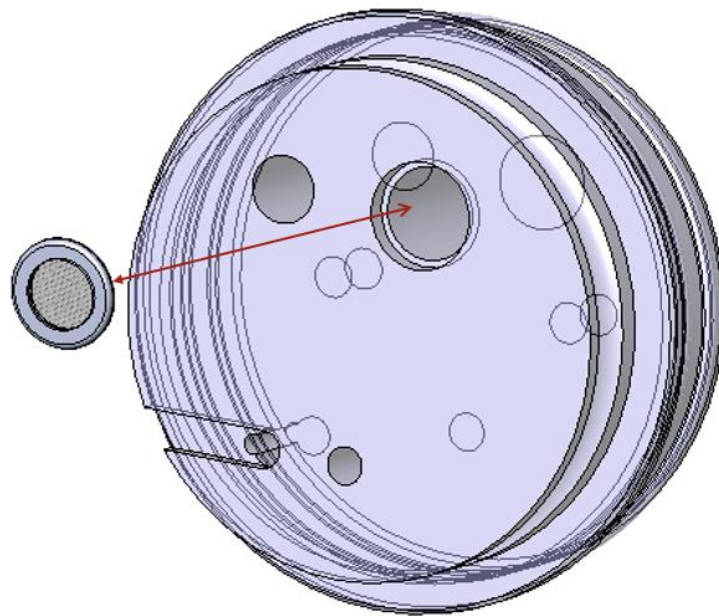
- Silicone Lubricant Use to lubricate chamber and restraint O-rings. 5.3 oz tube
- Pick Tool Use to help remove O-rings.
- Syringes (10cc) Use to push/pull air into the chamber for system testing.
- Syringe Tubing Assembly Use to connect syringe to calibration plug.
- Calibration Plugs Calibration plugs used to seal the neck seal during calibration (3 sizes – S, M, L), includes all required fittings and tubing.

PNEUMOTACH SCREENS

This section details the process used to replace pneumotach screens. These fine mesh screens, mounted in the silicon plethysmographs rear cap of the headout chamber provide the stable resistance elements necessary to accurately monitor the breathing waveform of the animal. The resistance element is part of the transducer/preamplifier circuit that, when calibrated, provide a waveform that the software analyzes during the course of the experiment. When these screens become contaminated due to environmental dust and ordinary usage over an extended period, the resistance provided by the screens will change. As the resistance of the screens changes, the effective range of the chamber will change.

SCREEN REPLACEMENT

To replace the pneumotach screens, gently pop it out of the silicone plethysmographs rear cap and either clean it or replace it them with a new screen. The screens are made from stainless steel and are very robust. If properly handled the screens can be washed numerous times before being replaced. If the screen is washed ensure it is through dried before replacing it back into the rear plethysmographs rear cap. Two replacement screens are included with the chamber maintenance kit.



CHAMBER MAINTENANCE KITS

A chamber maintenance kit is available for each size chamber.

- Replacement kit part number: 601-6601-001 (Small Head-Out maintenance kit)
- Replacement kit part number: 601-6601-002 (Medium Head-Out maintenance kit)
- Replacement kit part number: 601-6601-003 (Large Head-Out maintenance kit)

The maintenance kits consist of the following items:

- 8 – Replacement O-rings
- 25 – Silicone Plugs (used for plugging Accessory Port on rear cap)
- 2 – Replacement Pneumotach Screens
- 1 – Tube Silicone Grease, 5.3 oz, food grade

Note: Kits contain additional components that are not discussed in this section. Components may differ based on species and the type of chamber used.

- Silicone Lubricant part number: 601-2524-052

CLEANING THE CHAMBER

Clean animal chamber of debris as needed by wiping with a cloth or rinsing with warm water. For a more thorough cleaning, use warm water and a mild dish detergent. It is best to let the chambers thoroughly air dry. If required, dry with a soft cloth towel.

For disinfecting post cleaning, Rescue wipes and or ready to use Rescue spray (0.5% Hydrogen peroxide as the active ingredient) has been found to be an appropriate disinfectant. (<https://www.viroxanimalhealth.com/>).

NOTE: The Polycarbonate tubes used for this chamber have high tolerance to impact and higher temperature tolerance than many other materials often used in chamber designs. Polycarbonate does have a limited resistance against alkaline chemicals such as ammonia and stronger incompatibility towards aromatic hydrocarbons and chlorinated hydrocarbons. Use of strong alkaline cleaners will cause crazing and cracking of polycarbonates.

- **DO NOT USE WINDEX** or window cleaners to clean the plethysmograph. Continued use of such cleaners will eventually cause crazing and cracking of the polycarbonate material.

PLEASE consult with DSI for advice related to compatible machine-washing temperatures and detergents.

TECHNICAL SUPPORT

DSI™ is available to help you with your questions and concerns. Should you hit a road block or need some additional training, please feel free to contact us. We are happy to help!

DSI TECHNICAL SUPPORT—NORTH AMERICA

Email: Support@datasci.com

Toll-free in U.S. and Canada

Phone: 1-800-262-9687

Monday through Friday: 8 AM to 5 PM CST
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APPENDIX

CHAMBER DIMENSIONS

Chamber	Inner Length Min - Max	Overall Length Min - Max	Restraint Tube Diameter	Rear Tube Diameter
601-3921-001 Small (125-200g)	5.5" – 6.5" (14.0cm – 16.5cm)	8.7" - 9.7" (22.1cm - 24.6cm)	2.50" (6.35cm)	3.00" (7.62cm)
601-3921-002 Medium (200- 400g)	6.5" – 8" (14.0cm – 20.3cm)	9.7" - 11.2" (24.6cm - 28.4cm)	3.00" (7.62cm)	3.50" (8.89cm)
601-3921-003 Large (400-600g)	7.5" – 10.9" (19.1cm – 27.7cm)	10.7" – 14.1" (27.2cm – 35.8cm)	3.50" (8.89cm)	4.00" (10.16cm)

