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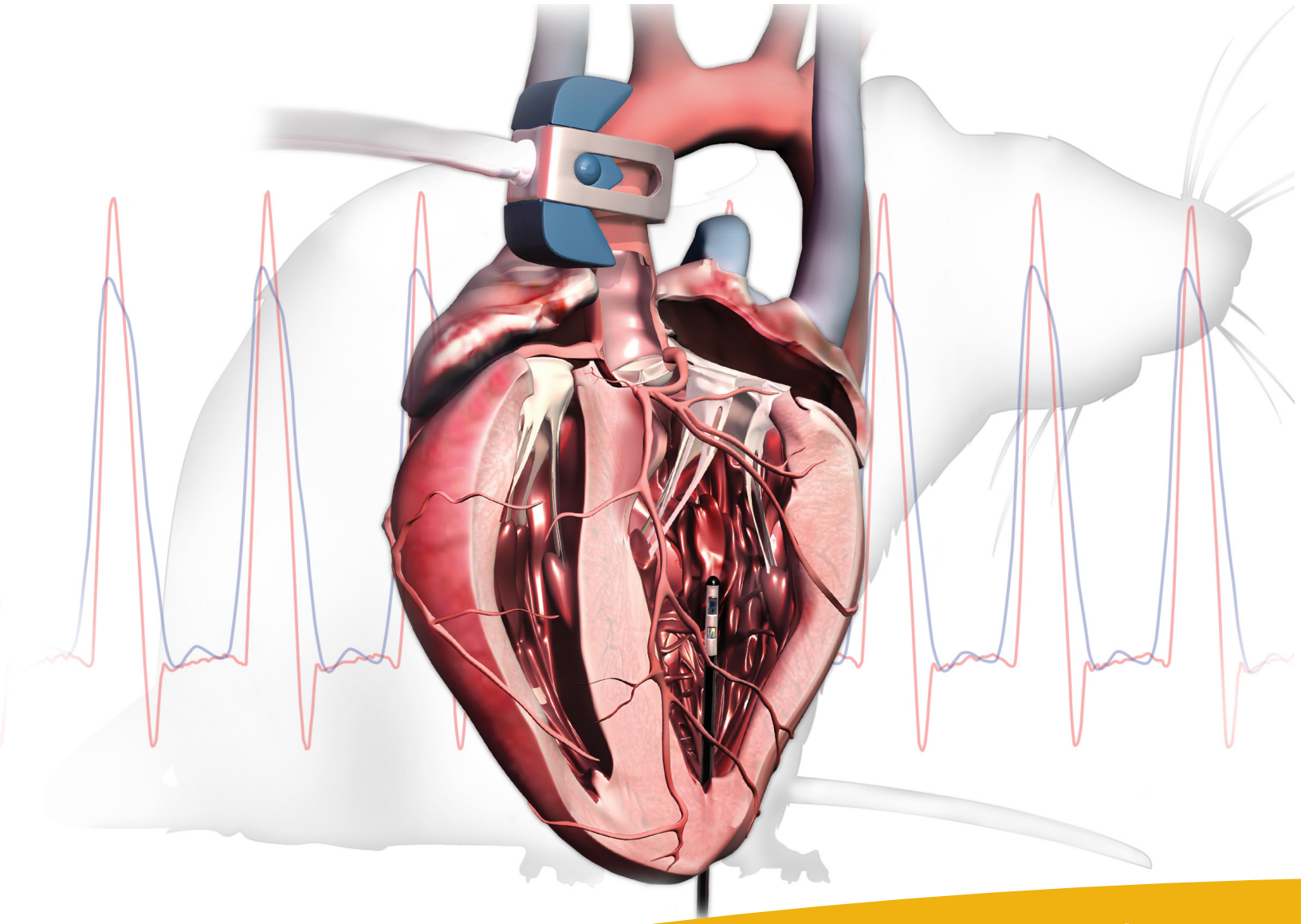
THE MEASURE OF BETTER RESULTS.

The Guide to Acute Pressure and Flow Measurements in Rodents



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This workbook presents protocols, data tables and graphs, tips of the trade and application support for successful hemodynamic measurements using pressure and transit-time volume flow technology. It is designed to be a comprehensive guide for pressure and transit-time flow studies. Note: appropriate surgical procedure, accurate collection and interpretation of data is the responsibility of the researcher.

This workbook is a living document that will change over time due to technological improvements, new surgical approaches and the exploration of new applications. Nevertheless, we believe that this workbook, in its current form, will serve to advance the measurement of better results for you.

We appreciate the feedback of our many customers whose studies form the foundation for the included application protocols and whose quest for solid scientific data continues to stimulate ongoing product improvements.

This handbook is an educational service provided by Transonic Systems Inc.®,
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Copies of this handbook may be ordered from Transonic Systems Inc.® or its distributors (contact information below).

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Transonic Systems Inc. is a global manufacturer of innovative biomedical measurement equipment. Founded in 1983, Transonic sells "gold standard" transit-time ultrasound flowmeters and monitors for surgical, hemodialysis, pediatric critical care, perfusion, interventional radiology and research applications. In addition, Transonic provides pressure and pressure volume systems, laser Doppler flowmeters and telemetry systems.

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Introduction

Dear Fellow Researcher,

Thank you for trusting Transonic's resources and technology to conduct your research. We have created this workbook to be a comprehensive guide to using our pressure and transit-time volume flow measurement systems in small animal studies. This should be seen as a companion piece to the manuals and other workbooks to help integrate our products into your laboratory and experiments.

The information contained within comes from the extensive experience of Transonic's staff and research collaborators. We appreciate the feedback of our many customers whose studies form the foundation for the included application protocols and whose quest for solid scientific data continues to stimulate ongoing product improvements.

We will start with reviewing Transonic's pressure equipment, how to work with and take care of the equipment. We will subsequently do the same for our transit-time volume flow meters and probes. Next you will find anesthesia guidelines, equipment recommendations and more for your work in rodents (mice and rats), as the quality of your data will improve with the correct preparations. Following this we will review several applications. Some are pressure only, others benefit from both pressure and volume flow measurements. For each application we will explain how to perform the surgery in the form of a detailed protocol and show you example data, so you know what you can expect. Finally, we will share protocols for setting up data collection in several common software programs. Our hope is that this workbook will provide an useful guide for developing your own protocols. Of course, accurate collection and interpretation of data is the responsibility of the researcher, but you are always welcome to contact your local Transonic representative for support and guidance.

This workbook is a living document that will change over time due to technological improvements, development of new surgical approaches, or its applications. Nevertheless, in its current submission we believe that this Workbook will serve to advance the measurement of better results.

Sincerely,

The Transonic Research Team

FOR A COMPILATION OF ACUTE AND CHRONIC TECHNIQUES USED IN RODENTS TO STUDY BLOOD FLOW IN VARIOUS MODULES SEE:

Tools & Techniques for Hemodynamic Studies in Rodents Workbook

FOR A COMPREHENSIVE GUIDE TO PRESSURE-VOLUME TECHNOLOGY AND PRACTICES SEE:

Tools & Techniques for Pressure-Volume Hemodynamic Studies Workbook

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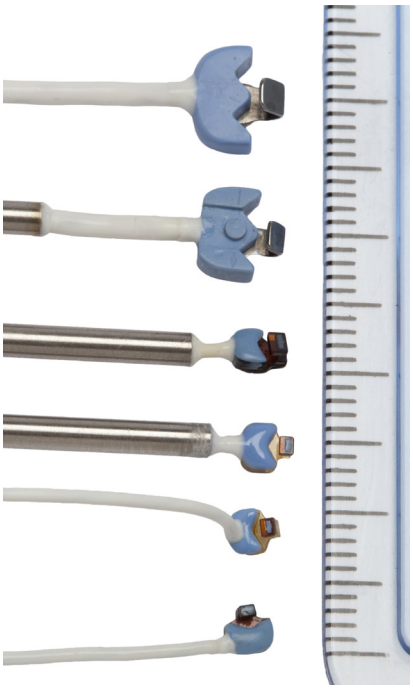
Measurement Tools & Best Practices



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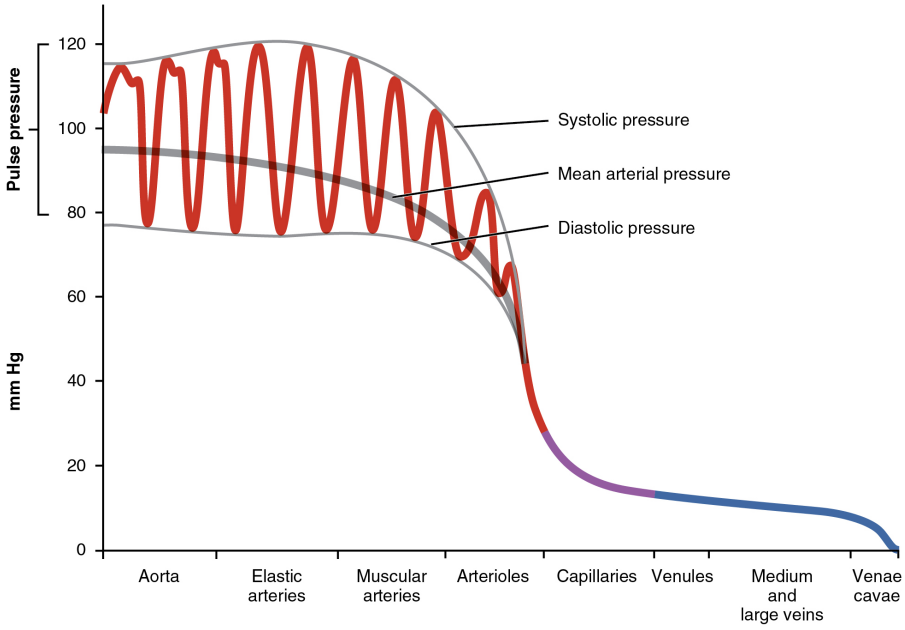
Introduction to Invasive Pressure Measurements

INTRODUCTION TO INVASIVE PRESSURE MEASUREMENTS USING SOLID STATE GAUGE PRESSURE SENSOR

Most scientists know that blood pressure is expressed in millimeters of mercury (mmHg). Not everybody knows that the reason for this is that the physician-physicist Jean Louis Marie Poiseuille (1799–1869) was the first to experiment with a so called "hemodynamometer." This device was a mercury filled U-tube manometer connected to a cannula filled with potassium carbonate (used as an anticoagulant). The cannula was inserted directly into an artery of an experimental animal (1). Poiseuille was also able to demonstrate that arterial pressure is maintained in the small arteries up to 2mm, and that blood flow though mesenteric capillary bed was not directly dependent on venous pressure changes, rather influenced by arterial pressure changes. This was an important step forward, contributing to the formulation of what we now call Poiseuille's law (2).

In the modern era, invasive blood pressure measurements can be performed using wide variety of devices. Two commonly used devices to measure arterial blood pressure are the fluid filled pressure transferring catheter with a pressure sensor located externally to the body, and the direct measurement inside the artery with a miniaturized catheter-based pressure sensor.

"Blood pressure" is the pressure of circulating blood on the walls of blood vessels. It is a direct result of the contraction and build-up of pressure in the left ventricle of the heart. There is a strong relationship between blood pressure throughout the body, the diameter of the vessels the blood flows through and the distance to the left ventricle for instance.



High systemic blood pressure has been one of the leading risk factors for the overall global disease burden. High diastolic blood pressure for instance is strongly related to an increased risk of developing an abdominal aortic aneurism. Pulmonary artery hypertension is associated with decreased right ventricle function. The overall decrease in cardiovascular mortality in high-income countries is a direct result of fundamental and applied research in the past 30 years. As a result of this there is a rise in the number of patients living with cardiovascular disease. This makes cardiovascular disease an imporant area of ongoing research. Chronic high blood pressure is not only linked to heart disease, it is also the second leading cause of kidney failure. Applications and supportive documents directly related to this research field can be found in this workbook.

We are going to start with introducing Transonic Scisense pressure equipment and how to handle and take care of the catheters. All with the aim to measure better results the Transonic way.

REFERENCES:

(1) Booth J. A short history of blood pressure measurement. Proc R Soc Med 1977;70:793–9

(2) Sutera SP, Skalak R. The history of Poiseuille's law. Annu Rev Fluid Mech. 1993;25:1–19.

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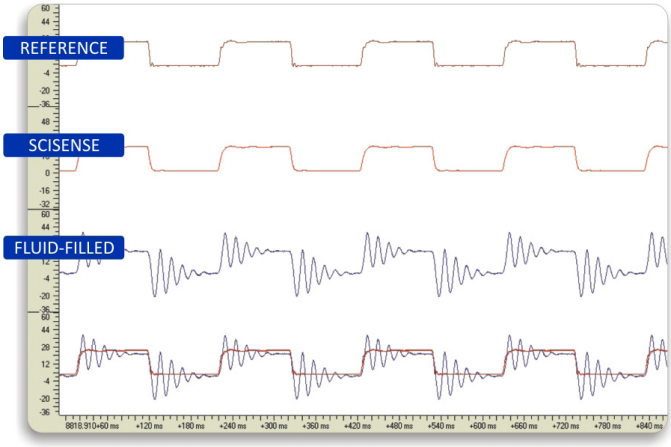
Transonic Scisense Pressure Systems & Catheters



Transonic Scisense Pressure Catheter compared to a reference trace and a fluid-filled pressure catheter at 300 BPM (5 Hz). The fluid-filled catheter shows large distortion and wave artifact compared to the Transonic Scisense trace which reflects the true pressure waveform of the reference sensor.

SP200 PRESSURE MEASUREMENT SYSTEM

- Measure two channels of pressure simultaneously
- Permits calculation of pressure gradients and pulse-wave velocity
- Quick electronic two-point calibration
- Compatible with all data acquisition systems (± 5V range required)

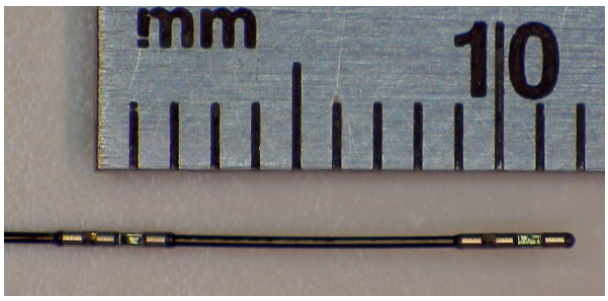
Single sensor 1.2F Pressure Catheter

Single sensor 1.6F Pressure Catheter

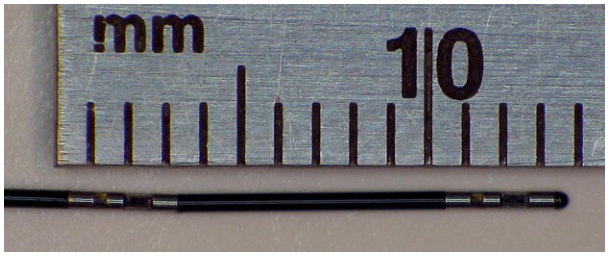
SOLID-STATE PRESSURE CATHETERS

Single pressure catheters are catheters with one pressure sensor on it. They are used when one is interested to measure the pressure at a specific location. Dual pressure catheters have two separately operating pressure sensors on the same catheter. They are used for pulse wave velocity measurements. The length in between the two pressure sensors is determined by the distance between the two loci of one's interest.

- Single and dual pressure sensor catheters are available in all sizes ranging from 1.2F to 7.0F.
- High frequency response ensures accurate detection of pressure waveforms and resulting calculations (dP/dt, Peak Pressure, MAP, etc.).
- Variable placement of second pressure sensor on dual catheters is available to meet protocol needs.
- Catheter tips can be customized to enable easy insertion.
- Smooth, flexible tubing allows easy insertion and navigation.



Dual sensor 1.2F Pressure Catheter



Dual sensor 1.6F Pressure Catheter

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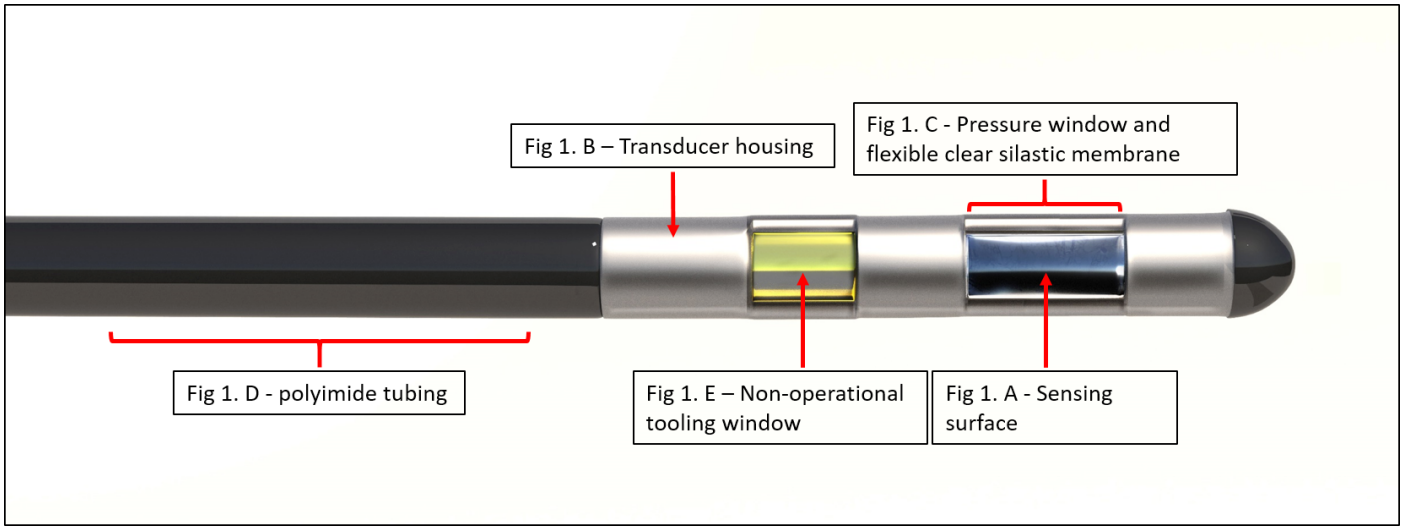
Anatomy of a Pressure Sensor

Pascal's law states that for a fluid in a closed container, a pressure change in one part is transmitted without loss to every portion of the fluid and to the walls of the container.

When inside a ventricle, the catheter's "sensing surface" (Fig 1. A) will experience the pressure of the fluid within the chamber regardless of where the sensor is inside the chamber. This sensing surface is a small portion on one side of the "transducer housing" (fig 1. B) and is able to sense pressure through a small opening in this housing. The opening is referred to as the "pressure window" (Fig 1. C). The pressure window is located directly above the transducer sensing surface. The pressure window is filled with a "flexible clear silastic membrane" (Fig 1. C) that is in simultaneous contact with the transducer sensing surface and the blood. The flexible membrane is responsible for separating the transducer electronics inside the housing from moisture and also for transferring the force of the blood pressure to the transducer sensing surface inside the sensor housing. This along with the external "polyimide tubing" (Fig 1. D), and delicate internal wires that transmit the recorded data, constitute a **solid-state pressure catheter** (Fig 1).



Things to be aware of when using micro pressure sensor catheters:

PRESSURE SENSOR ORIENTATION

In order for the transducer to work, the pressure window must be exposed to the body of fluid being observed. Keep in mind that the sensor window is on the side of the sensor. When the pressure window interacts with tissue rather than fluid, the result will be the sensor reporting the force of tissue interaction, and not the fluid pressure of interest. Situations where the transducer is reporting tissue force is when the transducer is in a small diameter blood vessel and interacts with the vessel wall or in a ventricle and the sensor window interacts with trabeculae or ventricular walls.

Anatomy of a Pressure Sensor Cont.

SHORT TERM FLEXIBLE MEMBRANE CHANGES

The flexible silastic membrane that separates the sensing surface from fluid must be largely impermeable to fluid. In reality all silastic materials have some degree of permeability, meaning that the flexible membrane absorbs some amount of fluid and swells when exposed to water. The swelling will change the dimensions of the flexible membrane and exert force on the sensing surface. The changing output from the sensor during this event is called "Hydration drift". Hydration drift will continue until the silastic has absorbed as much water as it can; typically half an hour in a saline solution. This is why we hydrate our catheters prior to use.

LONG TERM FLEXIBLE MEMBRANE CHANGES:

Inevitably, the mechanical properties of the silastic sensing membrane are going to change with time and use. A small part is due to age and ambient environment when the catheter is stored. A larger influence is care and maintenance of the sensor post-use. Dissolved proteins entering the silastic membrane during use and any tissue left to dry on the flexible membrane can alter its mechanical properties over time. Much can be done to lessen any degradation if the catheter is not allowed to dry with any bio-material on the membrane. Careful adherence to the cleaning steps as outlined in our care and use documents will prolong catheter life and accuracy.

BALANCING THE TRANSDUCER

Before using a pressure catheter, the sensing surface needs to be balanced or "Zeroed". This is an electronic method to compensate for any system changes in the sensor or its electronics; either as it ages or undergoes thermal or re-hydration driven forces. Transducer balancing must be done before every experiment. This process is covered in our Quick Start Guides and user manuals.

MECHANICAL COMPONENTS INSIDE THE CATHETER:

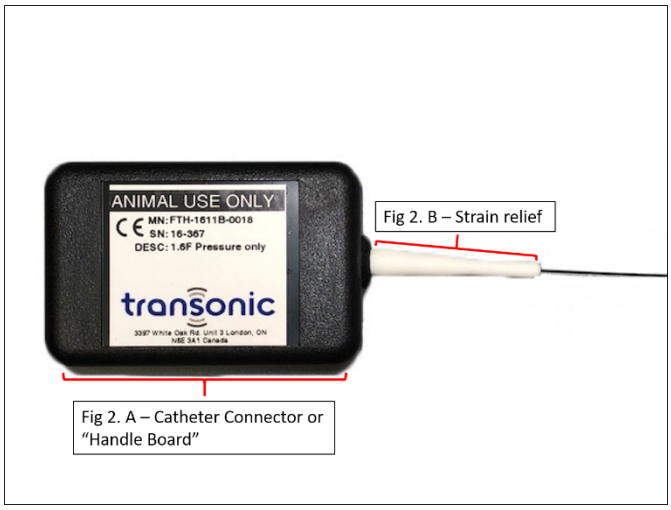
In order to prolong the life of a micro catheter, it is important that the user have some appreciation of the mechanism. The sensing surfaces, tube wall and conducting wires are measured on the order of microns. It is not unrealistic for an untrained user to unknowingly apply damaging forces to the catheter.

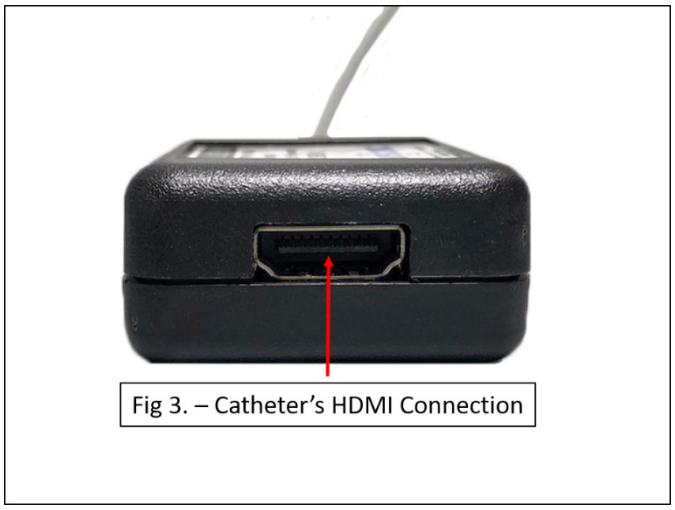
1. A common form of damage to micro tip catheters is catheter shaft damage from excess force applied to the shaft. Similar to an eggshell, the polyimide shaft material will collapse if it is deformed past a certain point either by bending or crushing. Shaft strength will not return once it has gone past the yield point. The catheter shaft will also be weakened by any micro cuts or abrasion. Collapsed shaft tubing is often responsible for damaging the very small wires inside the shaft.
2. The transducer sensing surface is only microns thick and resides directly under the flexible membrane in the pressure window. It is important that any non-fluid forces be minimized in this area. Tissue, bending and abrasion from overly tight sutures are examples of forces that can rupture the sensing surface.

Anatomy of a Pressure Sensor Cont.

CATHETER CONNECTOR AND STRAIN RELIEF

To this point we have talked largely about the catheter's distal tip, but consideration must also be given to the catheter's connector and strain relief. Located at the proximal end of our catheter is a connector or "handle board" (Fig 2. A), which allows for interface with our pressure hardware via an "HDMI connection" (Fig 3.). This area should be kept dry and free of dust/debris at all times to ensure proper functionality. Directly attached to the handle board is a white rubberized "strain relief" (Fig 2. B) that is designed to reduce stress on the polyimide tubing that can be caused by articulation of the tubing relative to the handle board. We suggest minimizing the necessity of the strain relief and this can be accomplished by positioning the handle board close to your subject and taping it down. This will prevent unexpected movement of the connector that can happen during a complex surgery. With the above fundamental information in mind, a careful review our handling and care documentation will go a long way to prepare you for successful and safe data collection.



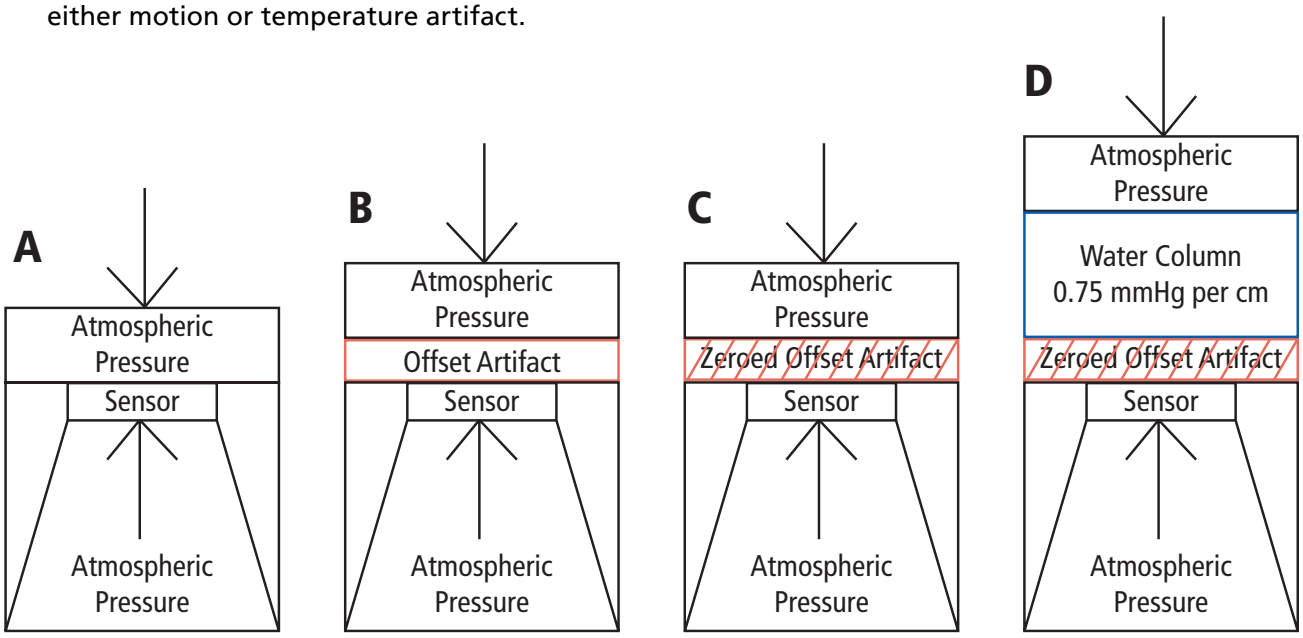


Balancing Pressure Sensors Before Use

Transonic Scisense Pressure Catheters are solid state pressure catheters built with piezo resistive strain gauges that detect pressure through a flexible rubber membrane. Due to the mechanical properties of the rubber and the nature of gauge pressure sensors, correct use requires an understanding of how to properly balance the sensor.

BALANCING YOUR CATHETER BEFORE USE:

1. Air Calibration: Calibrating the Sensor in air should provide the best result because there is no excess pressure exerted against the membrane. There are, however, two considerations:
   a. Since the catheter should have been soaked in fluid for some time before use, it will be wet when exposed to air. The exothermic effects of evaporation could exceed the temperature compensation features that are built into the catheter.
   b. Since the catheters are very sensitive, any motion might be detected when holding the catheter. Combined with exothermic events, this can result in a wandering signal. If the software used to analyze the signal is in an auto-gain mode, the effect is exaggerated even further.
2. Calibrating the Sensor in saline: Body temperature saline used to soak the catheter is the best environment for catheter calibration. However, the user needs to be aware of the offset value that a saline column will create. If the catheter is zeroed under a 5 cm column of saline when it is removed from the water, the reading will be -3.5 mmHg. Inserting the catheter into a ventricle with this offset would result in a negative EDV value. The best way to calibrate the catheter is to hold it just under the surface (meniscus) of body temperature saline as it is being balanced. The offset should be minimal for a catheter under a few mm of water. Any minor signal wandering as the catheter is transferred to the blood vessel can be ignored as either motion or temperature artifact.



(A) Ideal Pressure Sensor referenced to atmospheric pressure. The forces on both sides of the sensing membrane balance so the output is zero.
(B) In the real world, there is always a mechanical or electrical factor that is going to cause an imbalance across the pressure sensing membrane. The artifact will vary between catheters and associated amplifiers.
(C) For this reason, each control box comes with an offset correction control which can be used to counter balance the offset artifact. This electronically zeros the output.
(D) The Sensor can then be submerged in a beaker of water to a given depth. Since the artifact has been cancelled out and the atmospheric pressure is equal on both sides, the Sensor will output 0.75 mmHg for each centimeter of water depth it is submerged.

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Basics Behind the Types of Pressure Sensors

To put the Transonic Scisense pressure catheter in a bigger picture, we would like to introduce you to the variety of pressure catheters available.

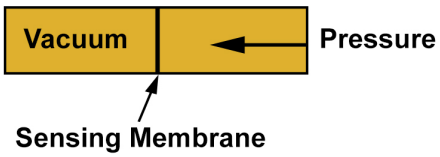
Pressure Sensing Technology Basics

- Pressure can be converted to some transitional form of displacement.
- The sensor converts this displacement into an electrical output such as voltage or current.
- The four most universal types of blood pressure transducers are the piezoresistive strain gauge (Transonic Scisense pressure catheters are of this type), variable capacitive, piezoelectric, and optical pressure transducers.
- Wheatstone bridge (strain based) sensors are the most common pressure sensor types.
- During pressure change a sensing membrane diaphragm is displaced, and an equivalent change in resistance is induced on the strain gauge, which can be measured. Most strain based pressure transducers output a small mV voltage.
- Bridge sensors are used for high and low pressure applications, and can measure absolute, gauge, or differential pressure depending on the sensor configuration.
- Pressure sensors generally produce a linear response across the working range of the transducer.
- Linear response of a pressure transducer is when the transducer outputs a conditioned current (e.g. 0-10 V signal or 5-20 mA), where both 0 V and 5 mA might correspond to a 0 mmHg pressure. Likewise, linear 10 volts and 20 mA correspond to the full scale capability or the maximum pressure the transducer can measure.
- Later, software converts the voltage output of the sensor into a pressure measurement. Starting on page 123 of this book, you will find documents on how to set-up commonly used data acquisition software programs in laboratory research.

Types of Pressure Sensors

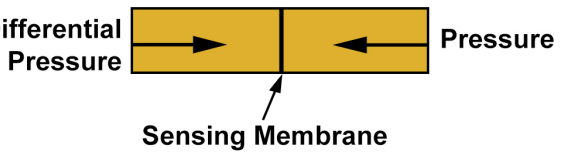
ABSOLUTE PRESSURE SENSOR:

The device measures pressure relative to internal reference vacuum.



DIFFERENTIAL PRESSURE SENSORS:

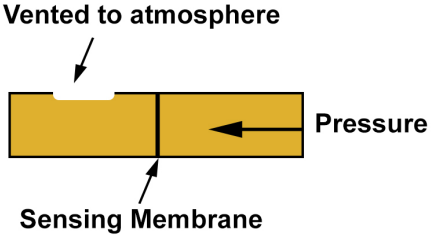
The device measures difference between two pressure measurements placed on the same sensing structure. Instead of measuring relative to atmospheric pressure, differential measurements are taken with respect to a specific reference pressure.



GAUGE PRESSURE SENSOR:

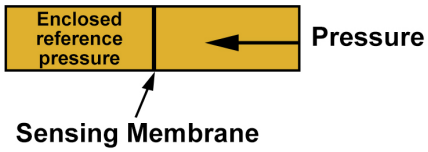
The device measures relative to atmospheric pressure, part of the sensor must be “vented” to atmosphere.

In negative pressure region vacuum gauge sensors might be used that are a form of gauge pressure sensor. The device would show the difference between negative pressure and atmospheric pressure.



SEALED PRESSURE SENSORS:

The device senses pressure relative to some permanent reference pressure inserted before sealing (It functions as a differential pressure sensor).



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Review of Validation Paper of Scisense Pressure Sensor

Catheter tip pressure sensors are not unique to Transonic Scisense; they were in fact pioneered by Huntley Millar decades earlier and used for rodent studies since the late 1990's. Transonic Scisense pressure catheters have, however, proven comparative accuracy and reliability as a new-comer on the market as referenced in this validation. Our focus is to provide expert research support and service as we have for our flow measurement devices, and continue to excel in the application for our pressure sensing technology.

METHODS

- A total of nine 1.4F Millar and eleven 1.2F Scisense Catheters were used for all experiments. Each protocol used between 3 -6 Catheters of each brand.
- *In Vitro* assessments were made for Temperature Drift and Frequency Response up to 250 Hz.
- A Pop Test was used to determine natural frequency and damping coefficient.
- *In Vivo* Isoproterenol Dose Response and Dobutamine Dose Response tests were used to assess dP/dt performance.
- Pressure Drift post intervention was examined over a 45 minute period.
- Simultaneous Left-Ventricular Pressure Measurements were taken from both catheters in the same LV during steady state, transient occlusion of the inferior vena cave, aortic pinch and arrhythmias.

RESULTS

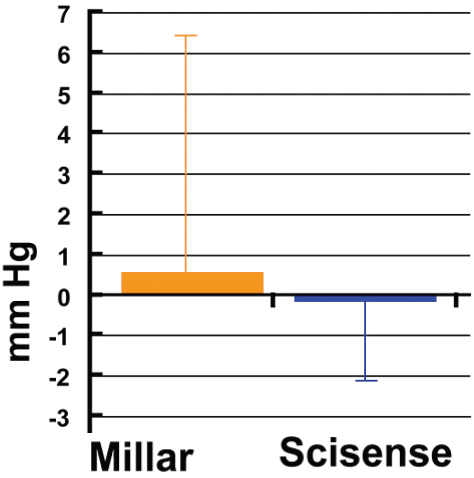
- There was no significant difference in Frequency Response or the amount of Temperature Drift, though Millar tended to overestimate pressures while Scisense underestimated pressures.
- The natural frequency and damping coefficients as determined from the Pop Test were not significantly different.
- There was no significant difference in how the catheters measured + dP/dt or -dP/dt in either dose response study.
- The pressure drift over 45 minutes was very low and not significantly different between the Catheters.
- There was no significant difference in the measurements of left ventricular peak systolic pressure, left ventricular end-diastolic pressure, +dP/dt, or -dP/dt for any condition.

CONCLUSIONS

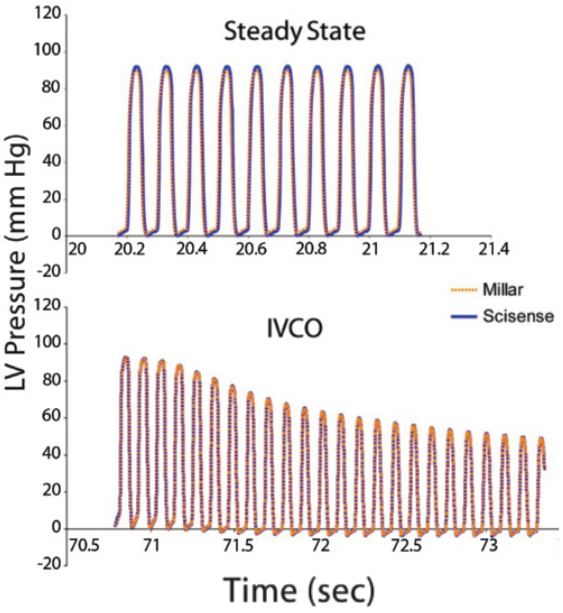
"We conclude that both sensors are equivalent, and that the Scisense pressure sensor represents an alternative to the current gold standard, the Millar micro-manometer pressure sensor for *in vivo* pressure measurements in the mouse."

REFERENCE

Trevino RJ, Jones DL, Escobedo D, Porterfield J, Larson E, Chisholm GB, Barton A, and Feldman MD "Validation of a New Micro-Manometer Pressure Sensor for Cardiovascular Measurements in Mice." Biomedical Instrumentation & Technology: Jan/Feb 2010, Vol. 44, No. 1, p. 75-83.



Pressure drift over 45 minutes for a Scisense Catheter and a Millar Catheter.



Simultaneous pressure measurements in a mouse LV by 1.2F Scisense Catheter and 1.4F Millar Catheter during steady state and IVC occlusion.

SP200 Quick Start Guide - Initial Setup

THIS IS ONLY A BASIC GUIDE.
PLEASE REFER TO THE USER MANUAL FOR COMPLETE OPERATIONAL INSTRUCTIONS.

1. Find a suitable location near work station or on roll cart (not provided). SP200 Pressure Amplifier Hardware and Data Acquisition System/Computer should be positioned close to the operator.

   Using the legend below and Figures 1 & 2 as a guide:

   - Connect power cable ① to Power Input ①
   - Connect BNC cables ② to Data Outputs ②
   - Connect HDMI cables ③ to Catheter Inputs ③

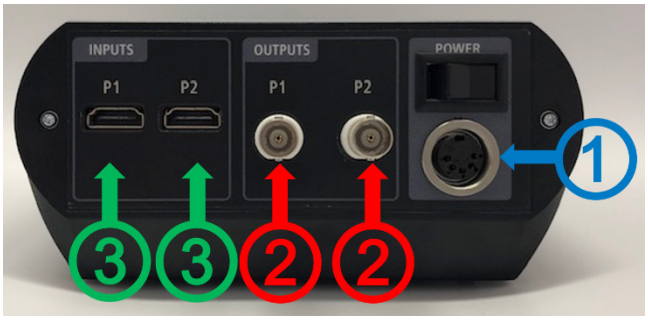


Fig 1. SP200 rear. Use in conjunction with Fig. 2

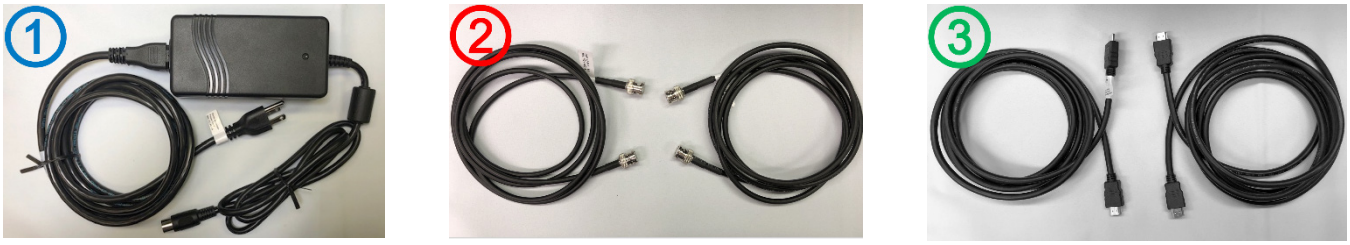


Fig 2. SP200 cables. Use in conjunction with Fig. 1

2. Using power switch on rear of SP200, turn on the system and verify that a green light beside the "MEA" button is illuminated (Fig. 3).
3. Connection to a Data Acquisition System (DAQ) can be made using the provided BNC cables (See ② in Fig. 2). The BNC cables output from "P1" & "P2" can connect to the channels of your choice on the DAQ. In Fig. 4 below, it is shown with P1 connected to Channel #1 and P2 connected to Channel #2 of the DAQ.

Fig 3. Measure button activated.

Fig 4. SP200 integration with DAQ

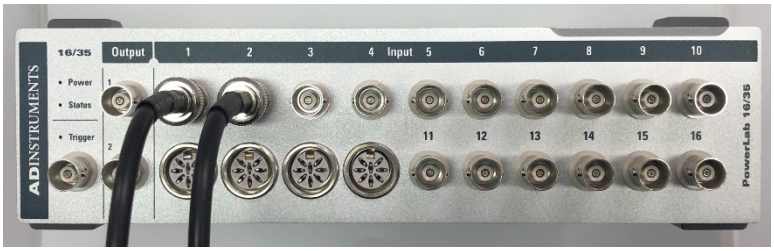


Fig 6. ADI PowerLab A-D Converter DAQ

transonic
THE MEASURE OF BETTER RESULTS.

SP200 Quick Start Guide - Initial Setup Cont.

4. With steps 1, 2 and 3 completed, you can now establish a "Template File" within your chosen software platform. Template files – also known as "settings files" – are software files that have specific calibration data saved for both output channels provided by the SP200. To complete this process, see below:
   a. For initial setup of a template file, locate the "START," "RECORD" or equivalent button in your software and select it – data should start to move across your screen.
   b. On the SP200, press the "0 mmHg" button highlighting it (Fig. 5) – wait for at least 3 seconds of data to scroll onto your software's screen. Repeat this process by pressing the "100mmHg" button highlighting it (Fig. 6) – again wait for at least 3 seconds of data to scroll across your software's screen. This will give you a low and high reference voltage on your software - corresponding to 0mmHg and 100mmHg respectively – that can now be converted within your software. Consult your DAQ's operating manual for more information related to voltage conversion.

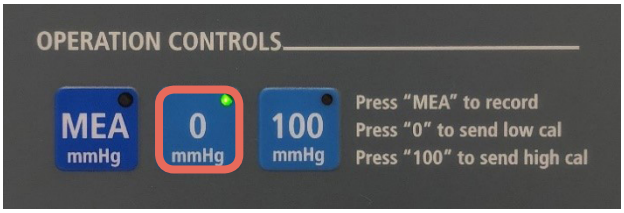
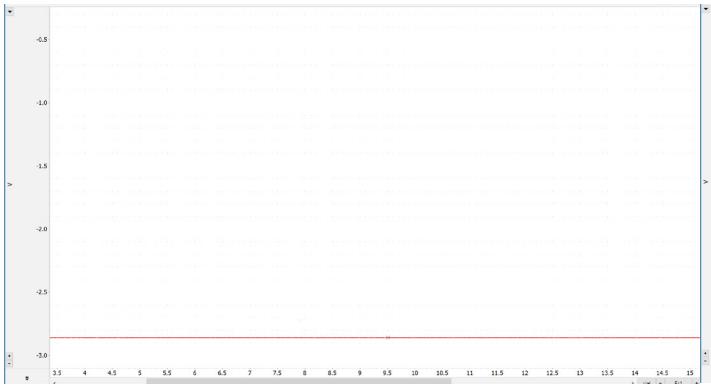
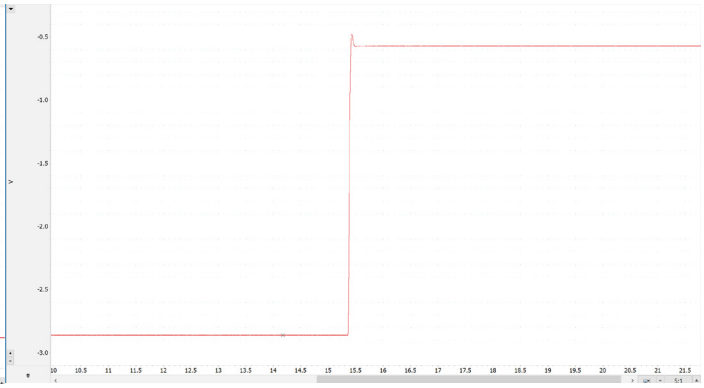


Fig 5. 0mmHg button and corresponding data output to software

Fig.6 100mmHg button and corresponding data output to software





   c. Confirm the sampling rate of your DAQ and adjust as needed. We suggest 1KHz for rodent models and 200Hz for larger animals.
   d. Once complete, it is essential that you save the settings of this file within your software for daily data recording. This will remove the need for daily equipment and software setup and stream line equipment use.

Setup is now complete, please reference our "Quick Start Guide – Daily Use Checklist" for a detailed review of our suggested daily use routine.

SP200 Quick Start Guide - Daily Use Checklist

Note: If equipment is not yet connected, reference "SP200 Quick Start Guide – Initial Setup"

1. Start Hydrating Distal Tip of the Catheter for 20-30 minutes

- The catheter tip must be fully submerged in fluid - body temperature saline is suggested when possible. 1.2-1.9F catheters can be placed into a filled 5ml-10ml syringe (needle removed) inserting the catheter through the luer-lock opening (Fig. 1). The catheter does not need to be plugged into the SP200 for this process.

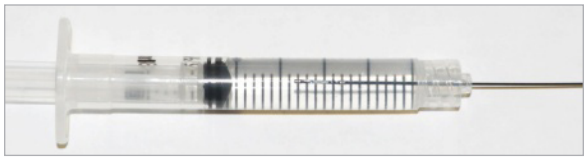


Fig. 1: Catheter hydrating in a syringe

2. Turn SP200 and Pressure Catheter ON

- Using the provided HDMI cables, plug one end of the HDMI cable into the catheter and the other end into the "P1" input on the back of the SP200. If using a second pressure catheter, repeat this process using the "P2" input. Power on SP200 using the rear switch - a green light beside the "MEA" button on the front will illuminate (Fig. 2).

Fig 2. Measure button activated.

- Note: "P1" has priority and should be used for any application where a single catheter is plugged in – this includes our dual-sensor catheters. Use "P2" only when two separate single-pressure catheters are in use.

3. Start Data Acquisition System (DAQ) and Load Template File

- Load your software program and select your pre-saved template file. Refer to "Step 4" of our "SP200 Quick Start Guide – Initial Setup" for more information.
- Once your template file is loaded, locate the "START," "RECORD" or equivalent button in your software and select it (Fig. 3) – data should start to move across your screen.

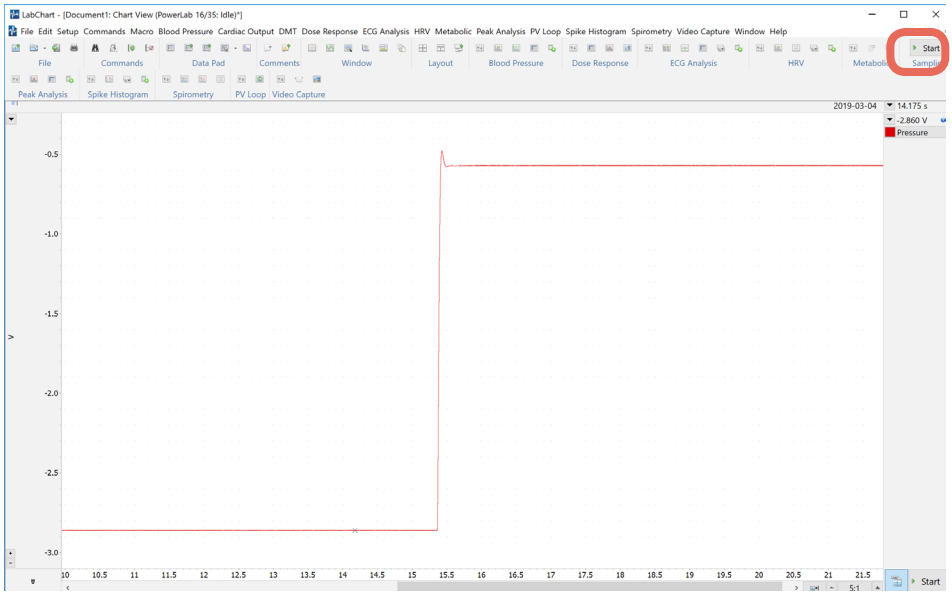


Fig 3. DAQ start in LabChart

SP200 Quick Start Guide - Daily Use Checklist Cont.

4. Balance Pressure Sensor

- After 20-30 minutes of hydration (see Step 1 above) – and with the catheter tip still submerged – raise the pressure sensor to just below the water line (Fig 4.).
- Press the "MEA" button, highlighting it - this allows for live recordings from the catheter. Then, using your DAQ software as reference, balance the recorded pressure to "0mmHg" using the "COARSE" and "FINE" adjustments buttons on Channel 1 of the Balance Controls section of the SP200 unit (Fig 5). Repeat process on Channel 2 if more than one pressure sensor is being used.
- Note: If you are not able to zero your pressure sensor, this may indicate that it is dirty (refer to our catheter cleaning guides) or that it requires service. Contact your Transonic representative if the problem persists.

Fig 4. Catheter balance below water line

Fig 5. Channel 1 pressure balance controls on SP200 are on top. Channel 2 pressure balance controls are on the bottom.

5. Collect Data from Area of Interest.

- Please refer to our "Optimizing Catheter Life Span" on page 17 for tips and tricks to extend the life of your catheter. A selection of surgical applications, including pictures and detailed instructions start on page 42.

6. Remove Catheter and Clean

- Proper catheter removal and cleaning are two key factors to catheter lifespan. Please follow all catheter handling and cleaning tips covered in our care guides – you play an important role in your life span of your catheter. Please contact your representative immediately if you have questions.

Cleaning Guidelines for Pressure Catheters

Due to their size, it is sometimes easy to forget that Transonic Scisense Catheters are highly technical and sensitive pieces of equipment. Proper movement of the pressure sensing window is essential. We recommend that you carefully examine your catheters when you receive them to get acquainted with their layout and store them in their supplied boxes when they are not in use. Abuse of the catheter due to mishandling or cleaning neglect will result in inaccurate measurements and reduced life span. The practice catheters included with each shipment are an excellent resource to help you become more familiar with proper handling and cleaning techniques.

- Always clean catheters immediately after each insertion.
- Use only approved products listed in Section 3; ultrasonic cleaners, alternative enzymatic cleaners or other products may damage catheters and void warranty
- Use this guide in conjunction with our "Optimizing Catheter Life Span" guide to ensure proper catheter handling.

1. Daily Cleaning and Care Guide

For all catheter cleaning applications, we recommend Endozime® AW Plus, a PH neutral enzymatic cleaning solution used to remove all traces of biological material. See Section 3 of this guide for more information about this product.

DAILY CLEANING GUIDE

Immediately after every insertion in blood, catheters should be cleaned. A 5-10ml syringe with the needle removed can be filled with pre-mixed Endozime® and the catheter inserted retrograde into the solution for convenient cleaning (Fig. 1). Be sure to submerge all parts of the catheter that were in contact with biological material. A soak of 30-90 minutes is normally sufficient but will depend on the amount of material on the catheter.

Fig. 1: Catheter in syringe with cleaner

Using Figures 2 & 3 as a guide, view the catheter under a microscope, if the catheter appears to remain soiled, soak the catheter further in Endozime® solution.

Fig. 2: Clean 1.2F Pressure Catheter

Helpful Tip: When multiple catheterizations are being performed throughout the day, carefully wipe with wet gauze any large contamination and place catheter in Endozime® between insertions. This step prevents tissue from drying onto the catheter that can affect measurement – the length of time for this soak is less relevant. Before reinsertion, fully rinse cleaner from the catheter as described in section 1.2 below and ensure that no contamination remains through visual inspection.

Fig. 3: Clean 1.6F Pressure Catheter

CATHETER RINSE

Catheters cannot be stored with cleaning agent on them, therefore a thorough rinse of the catheter is important. Using distilled water, carefully rinse catheter for 30 seconds either in a large basin moving slowly back and forth or under a gentle flow of distilled water. Ensure that the entire cleaned area is rinsed. Avoid fluid contact with the catheter's HDMI connection or strain relief.

Cleaning Guidelines for Catheters Cont.

DRYING AND STORAGE

Carefully dry the entire catheter length using soft, lint-free gauze taking extreme care around the pressure sensor on the distal end of the catheter. The pressure sensor should always be visible during the drying process to avoid accidental force being applied to it (Fig 4 & 5).

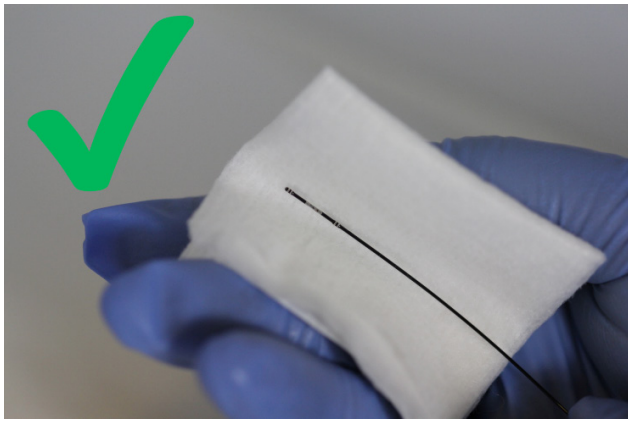


Fig. 4: Proper drying technique

Fig. 5: Improper drying technique

Perform a final inspection of the catheter, preferably under a microscope, to ensure no contaminants remain. If contaminates are found, repeat cleaning process above in subsection 1.1. The catheter can now be stored in original packaging. Ensure that the sensing portion of the catheter is centered in the circular cut out of the foam to avoid undue stress on the membrane (Fig. 6).

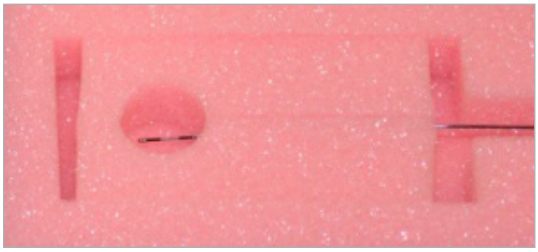


Fig. 6: Proper storage of catheter tip

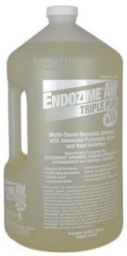
2. Disinfection Guide

Before disinfection, the catheter must be cleaned and dried as described above in Section 1.

- We suggest using CIDEX® OPA for disinfection, prepared in accordance with manufacturer's suggestion. See section 3 for specific product information.
- Submerge the catheter length to strain relief, being careful not to allow the fluid to make contact with the HDMI electrical contacts. Follow the manufacturer's prescribed submersion and temperature suggestions.
- Disinfectants will attach strongly to the catheter body, and therefore must be thoroughly rinsed before use. We suggest 2-3 minutes of rinsing and flushing with fresh and moving sterile solution (water or saline). Do not reuse flushed fluid and dispose of all fluids in a safe manner.

Cleaning Guidelines for Catheters Cont.

3. Recommended Products



Suggested Enzymatic Cleaning Agent:

- Endozime® AW Plus - Multi-Tiered Enzymatic Detergent
  Endozime® AW Plus is a unique low-sudsing and pH neutral formulation of enzymes that gently removes blood, fat, carbohydrates, starches and proteins. We suggest the unscented version with no perfume and no dye (Item #34516). Ordering information can be found at:
  https://www.ruhof.com/products/endozime-aw-plus?variant=1089952219150

Suggested Disinfecting Agent:

- CIDEX® OPA – Ortho-Phthalaldehyde Solution
  CIDEX® OPA Solution provides a broad-spectrum activity against bacteria, mycobacteria, viruses and fungi. More information can be found at:
  https://www.emea.aspjj.com/products/manual-solutions/cidex-opa-solution

4. Returning Your Catheter for Assessment

If your Transonic Scisense Catheter requires service, please contact us for return documentation. All catheters must be cleaned and disinfected before shipping to an authorized Transonic Scisense office for assessment. See sections 1 and 2 for cleaning guidance. Catheters that arrive with blood or tissue constitute a bio-hazard to our staff, therefore they will not be assessed, and the catheter will be returned to you at your expense.

For more information regarding service needs for your catheters or other equipment, please contact your local Transonic Scisense office, distributor or research sales representative for further guidance and assistance.

Our Transonic Scisense office can be reached at:

Transonic Scisense Inc.
3397 White Oak Rd., Unit 3
London, Ontario
Canada, N6E 3A1

Tel: +1 519-680-7677

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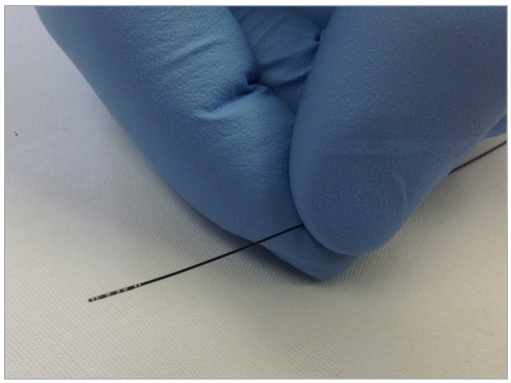
THE MEASURE OF BETTER RESULTS.

Optimizing Catheter Life Span

CATHETERS ARE DELICATE MEASUREMENT DEVICES: ALWAYS HANDLE WITH CARE.
FAILURE TO PROPERLY HANDLE CATHETERS MAY RESULT IN VOIDING THE WARRANTY.
FOLLOW ALL INSTRUCTIONS IN THE QUICK START GUIDES, INSERTS AND OPERATOR'S MANUALS.

In order to get the longest life possible from a Transonic Scisense Pressure Catheter, it is important that the user have some appreciation of the delicate nature of catheters. The sensing surfaces, tube wall and conducting wires are measured on the order of microns. However, when appropriate precautionary measures are taken, catheters can be successfully reused for many experimental protocols.

- Damage to the catheter shaft from excessive force is the most common cause of catheter failure. While the shaft material is very strong, it has a yield point, and will break if it is deformed past this point. The catheter shaft will also be weakened by any micro cuts or abrasion that may be inflicted during the course of its use.
  - Before handling a functional catheter, practice catheter handling and insertion with a dummy catheter.
  - If you chose to handle the catheter with forceps, place small pieces of polyethylene (PE) tubing over the forceps' tips. This will protect the catheter body against kinks or abrasions from the sharp forceps' edges. When using forceps to handle the catheter, please be aware that the forceps or grasping ends are not designed to manipulate such a delicate shaft.
  - When starting to work with catheters, please use a surgical microscope to estimate and coordinate the actual hand grip and applied strength under a variety of magnifications. This will help you with catheter/hand coordination and help determine the amount of force required to hold the catheter.
  - Ensure that you are not grasping the catheter with either fingers or forceps close to the area where metal rings or pressure sensor(s) are located. Applying pressure directly to the pressure sensor or metal rings can cause significant damage.
  - Even when using protected forceps, the user must be aware of the force generated on the catheter shaft. It should not be necessary to exert force to any extent that noticeably deforms the diameter of the catheter. Crushing the tube flat will destroy the strength and possible damage the wires inside. Note: This type of damage is easy to identify and will not be covered by the warranty.

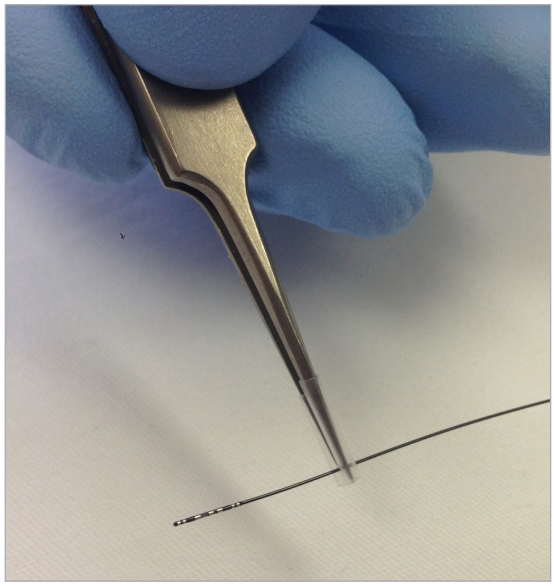


Correct catheter handling with fingers. Grip is along the shaft, well behind the sensitive catheter tip.

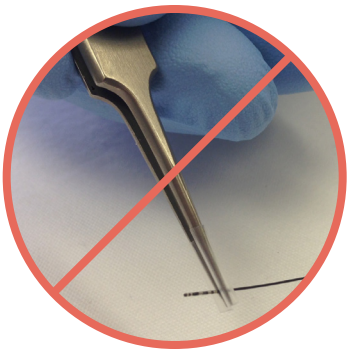
Place polyethylene tubing over the forceps' tips to protect the catheter body from damage.

Optimizing Catheter Life Span Cont.

- Be gentle when inserting and withdrawing catheters, especially when navigating past tie off sutures and heart valves, as this is the second most common way for catheters to sustain damage.
  - Consider using inhalation anesthesia to anesthetize the animal to ensure complete control over body position for the entire duration of the catheterization (from insertion to withdrawal). Any sudden change of position from an uncontrolled animal or anesthesia replenishment might damage (kink) the catheter.
  - When catheterizing a vessel, ensure the area where the catheter is inserted is well surgically prepared and maintained without an excessive amount of vascular sheets (adventitia). When placing a catheter in the ventricle or atria ensure that the pericardium is removed close to insertion site and that the appropriate sized needle is used to puncture the tissue. Never try to force the catheter through a thick layer of vascular adventitia or cardiac tissue.
  - If using a carotid artery access, the animal is usually in supine position, ventilated with 1-2% of Isoflurane, and all nociceptive withdrawal-reflexes are deficient. Insertion is through a well-cleaned segment of common carotid artery. Complete preparation and surgery can be seen on our website www.transonic.com. Access through the right carotid artery works better than the left carotid artery in LV catheterization (1).
  - The suture placed around the common carotid area and tied over the segment during surgery has to be positioned such that the sensing surface of the catheter does not bear direct contact with the suture. Moreover, it is important that holding and supporting sutures are not tied to the point where the catheter requires a strong force to pass through (during either insertion and withdrawal). Be careful, when tying off sutures, to stabilize the catheter during measurements. If the sutures are tied too tight, they can damage the catheter shaft.
  - When passing the catheter into the ascending aorta and through the aortic valve to enter the left ventricle (LV), the sensor tip of the catheter often encounters resistance at the valve entrance. If you are using a ventilation set-up, long supine-axis position can be adjusted to accommodate the catheter in such way that the catheter passes more in line with the supine-axis of the animal. This manoeuvre can be achieved by pulling on the front paws to reposition the animal, while slowly withdrawing and inserting the catheter without an excessive force. Trying to force the catheter past this point might result in the catheter bending too much and inducing a permanent crimp in the catheter shaft.



Correct catheter handling with forceps. PE tubing covers the forceps' tips and the grip is along the shaft, well behind the sensitive catheter tip.



Incorrect catheter handling with forceps. Note how the grip is on the sensitive catheter tip.

REFERENCE

(1) Migneco F, et. al. "New and simplified method for multiple left ventricle catheterizations in small animals." Interact CardioVasc Thorac Surg 2008; 7: 925-927.

Introduction to Ultrasonic Transit-Time Volume Flow Measurement

Blood flow refers to the movement of blood through a vessel, tissue, or organ, and is usually expressed in terms of volume of blood per unit of time (mL/min or L/min). It is initiated by the contraction of the ventricles of the heart. Ventricular contraction ejects blood into the major arteries, resulting in flow from regions of higher pressure to regions of lower pressure, as blood encounters smaller arteries and arterioles, then capillaries, then the venules and veins of the venous system. Blood supply is critical for life as it provided nutrients and oxygen to organs and tissue. Blood flow and blood pressure are strongly related, however measuring one does not automatically correlate to changes in the other.

The search for a better method for blood flow measurement was undertaken by Transonic founder Cornelis (Cor) Drost at the NYS Veterinary College at Cornell over 40 years ago. Working under the direction of Dr. Alan Dobson, professor of Physiology, Mr. Drost figured out how to measure the amount of blood flowing through blood vessels in a manner where one would not have to interfere with the flow inside the vessel itself.

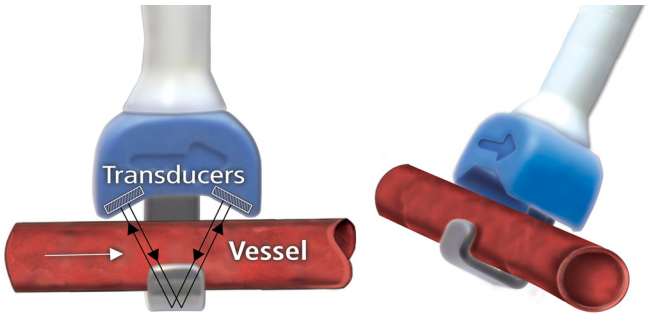
In 1978, the group presented this theoretical breakthrough to the world. The technology used differential transit time of upstream and downstream ultrasound signals to measure volume flow of blood directly from outside the vessel wall. Its revolutionary aspect was that it measured the actual volume of blood (rather than the blood velocity that Doppler systems measured) flowing through the vessel with high accuracy and without having to do things to the vessel that would change the very flow that one would want to measure. Transit-time ultrasound quickly became the gold standard for volume flow measurement due to its reliability, stable offset at zero flow and overall accuracy for in vivo blood flow applications in arteries, veins and even extracorporeal tubing.

Through collaboration with pioneering researchers, Transonic worked to miniaturize a small 1 mm flowprobe that could be used to measure renal blood flow in a rat. In 1990, the T106 and T206 Small Animal Flowmeters were introduced to the research community. By 1999, a tiny 1.5 mm mouse ascending aorta flowprobe was available; followed in 2000 by 0.7 mm and 0.5 mm flowprobes. Through Transonic's tradition of collaboration with pioneering research scientists, we have been able to address the ever challenging pursuit to resolve blood flow measurements with finer precision and accuracy and make those techniques available to the scientific community.

Researchers, by nature, demand more comprehensive and exacting methods in their studies to achieve significant results from their data - even more-so than the clinical studies that are built upon them. Many of the non-invasive clinical blood flow technologies available today have been validated in animal studies using Transonic research flowprobes as the gold standard, though our flowprobes are surgically invasive by design. However, simply measuring pressure – a clinically accessible parameter that is typically measured non-invasively - cannot give a complete hemodynamic picture. Both flow and pressure parameters are of great value and complement each other to give insight into disease states and research aimed to counteract these illnesses. It is thus important to include flow measurements in the research setting. We have therefore chosen to discuss both pressure and blood flow techniques in this book.

Transit-Time Ultrasound Technology Theory of Operation

A Transonic® Perivascular Flowprobe consists of a probe body which houses ultrasonic transducers and a fixed acoustic reflector. The transducers are positioned on one side of the vessel under study and the reflector is positioned at a fixed position between the two transducers on the opposite side. Electronic ultrasonic circuitry directs a Flowprobe through the following cycles:



Using wide beam illumination, two transducers pass ultrasonic signals back and forth, alternately intersecting the flowing liquid in upstream and downstream directions. The Flowmeter derives an accurate measure of the "transit time" it takes for the wave of ultrasound to travel from one transducer to the other. The difference between the upstream and downstream integrated transit times is a measure of volume flow rather than velocity.

UPSTREAM TRANSIT-TIME MEASUREMENT CYCLE

An electrical excitation causes the downstream transducer to emit a plane wave of ultrasound. This ultrasonic wave intersects the vessel under study in the upstream direction, then bounces off the fixed "acoustic reflector." It again intersects the vessel and is received by the upstream transducer where it is converted into electrical signals. From these signals, the Flowmeter derives an accurate measure of the "transit time" it takes for the wave of ultrasound to travel from one transducer to the other.

DOWNSTREAM TRANSIT-TIME MEASUREMENT CYCLE

The same transmit-receive sequence is repeated, but with the transmitting and receiving functions of the transducers reversed so that the flow under study is bisected by an ultrasonic wave in the downstream direction. The Flowmeter again derives and records from this transmit-receive sequence an accurate measure of the transit time it takes for the wave of ultrasound to travel from one transducer to the other.

Just as the speed of a swimmer depends, in part, on water currents, the transit time of ultrasound passing through a conduit is affected by the motion of liquid flowing through that vessel. During the upstream cycle, the sound wave travels against flow and total transit time is increased by a flow-dependent amount. During the downstream cycle, the sound wave travels with the flow and total transit time is decreased by the same flow-dependent amount. Using wide beam ultrasonic illumination, the Flowmeter subtracts the downstream transit times from the upstream transit times. This difference in the integrated transit times is a measure of true volume flow.

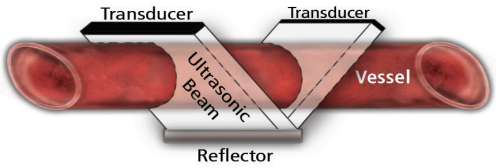
REFERENCES

1. Drost, C.J., "Vessel Diameter-Independent Volume Flow Measurements Using Ultrasound", Proceedings San Diego Biomedical Symposium, 17, p. 299-302, 1978.
2. U.S. PATENT 4,227,407, 1980.

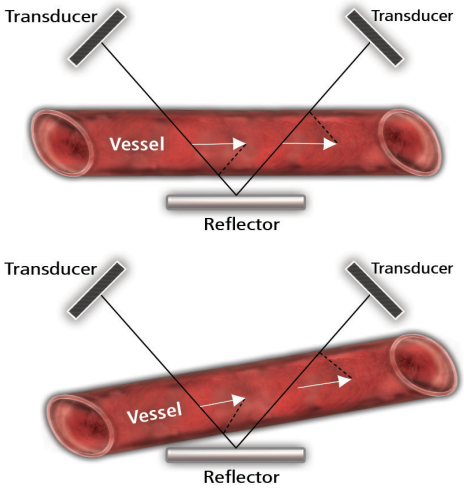
Transit-Time Ultrasound Theory of Operation Cont.

WIDE BEAM ILLUMINATION

One ray of the ultrasonic beam undergoes a phase shift in transit time proportional to the average velocity of the liquid times the path length over which this velocity is encountered. With wide-beam ultrasonic illumination, the receiving transducer integrates these velocity-chord products over the vessel's full width and yields volume flow: average velocity times the vessel's cross sectional area. Since the transit time is sampled at all points across the vessel diameter, volume flow measurement is independent of the flow velocity profile. Ultrasonic beams which cross the acoustic window without intersecting the vessel do not contribute to the volume flow integral. Volume flow is therefore sensed by Perivascular Flowprobes even when the vessel is smaller than the acoustic window.



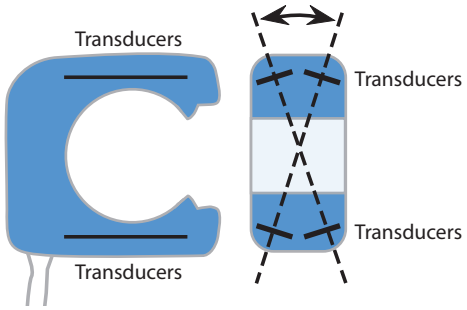
The vessel is placed within a beam that fully and evenly illuminates the entire blood vessel. The transit time of the wide beam then becomes a function of the volume flow intersecting the beam, independent of vessel dimensions.



The ultrasonic beam intersects the vessel twice on its reflective path. With each intersection, the transit time through the vessel is modified by a vector component of flow. The full transit time of the ultrasonic beam senses the sum of these two vector components. With misalignment (bottom), one vector component of flow increases as the other decreases, with little consequence to their sum.

X-BEAM ILLUMINATION

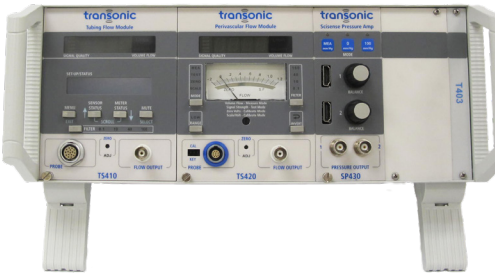
PAU-Series COnfidence Flowprobes® and XL Tubing Flowsensors use four transducers in X-beam illumination to accomplish the same volume flow measurements as the standard Perivascular Flowprobes. Ultrasound waves are transmitted in both the upstream and downstream directions by each pair of transducers. This provides two upstream and two downstream transit times which the Flowmeter combines into a single true volume flow measurement. The X-beam pattern of ultrasonic illumination provides the same advantages as wide beam illumination: measurement independence from velocity profile and vessel orientation.



COnfidence Flowprobes® use four transducers to create an X-beam ultrasonic illumination pattern to achieve a full vessel volume flow measurement.

400-Series Consoles & Modules for Research

Transonic's T402 & T403 Consoles allow mix & match Module capability in a single bench-top unit. The TS420 Perivascular Flow Module operates Flowprobes for in vivo blood flow measurements. The Flowprobes are configured for either acute/anesthetized or chronic/conscious protocols and are available for arteries, veins or ducts from 0.25 mm to 36 mm diameter. Inline and Clamp-on style Flowsensors are used on the TS410 Tubing Flow Module for volume flow measurements in tubing. The new SP430 Pressure Amplifier Module adds two channels of pressure using Transonic Scisense Pressure Catheters (or Transpac IV fluid-filled catheters). All modules output analog signals in the range of ± 5 volts ready for data acquisition.



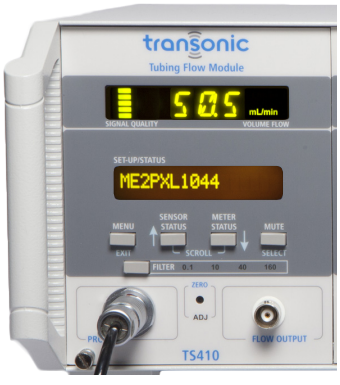
T403 Console with TS410 Tubing Flow Module and TS420 Perivascular Flow Module and SP430 Pressure Amp Module.

Transonic Gold Standard Flow Modules:

- Validated ultrasonic transit-time technology
- Direct volumetric blood flow measurement
- High resolution and zero baseline stability
- Continuous beat-to-beat flow data
- Non-constrictive Perivascular Flowprobes for vessels as small as 250 micrometers
- Inline extracorporeal Flowsensors for low flow isolated heart studies
- Solid-state Pressure Catheters for mice, rats and large animals.

TS420 Perivascular Flow Module measures volume flow in arteries, veins or ducts in laboratory animals.

SP430 is part of the T400-Series system and must be installed in a T402 or T403 console to function.



TS410 Tubing Flow Module measures volume flow of liquids in flexible plastic tubing.

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Transit-time Perivascular Volume Flowprobes

With over 35 years of experience in perivascular flow measurements, Transonic has a flow probe suitable for every blood vessel that is large enough to be isolated. All perivascular probes are compatible with the TS420 flow module. There are three different series of perivascular research probes available for rodent vessels. These probes are sized for a nonconstrictive fix on the vessel.

NANOPROBES

0.5-1.5 mm Nanoprobes are scaled to fit mouse anatomy for acute or chronic use.

PS- Nanoprobes

PR-SERIES

1 & 1.5 mm Flowprobes for small acute or chronic applications where a more robust design than the Nanoprobes is needed.

PR-Series

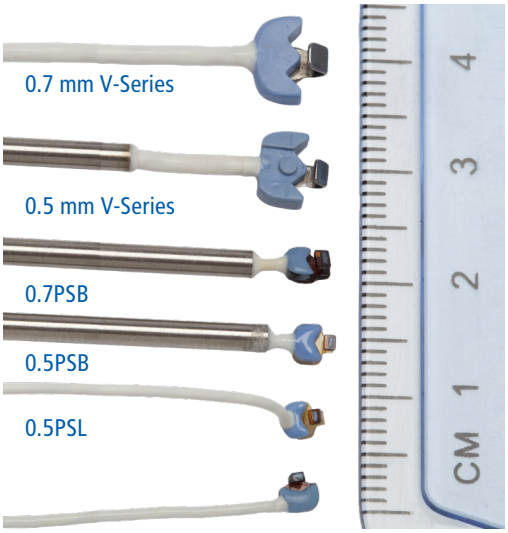
PS-SERIES

2-20 mm Flowprobes offer the greatest diversity of customizable features for the perfect fit in any application.

PS-Series

V-SERIES

0.5 & 0.7 mm Microcirculation Flowprobes for acute use only. Larger body and more robust than Nanoprobes. Requires more acoustic gel for the larger lumen.



Nanoprobe Care: 0.5PS & 0.7PS Nanoprobes

A Transonic Nanoprobe is a delicate instrument for precision blood flow measurements in microsurgical research applications. Please handle this instrument with precise care to enjoy full use and trouble free maintenance over the lifetime of the product.

HANDLING THE PROBES DURING USE

- Handle the chronic style (no handle) Nanoprobes by the cable just in back of the probe head, or by gently grasping the probe body. Never apply pressure or force on the reflector of the reflector hook, which is the most delicate part of the structure.
- Acute use Nanoprobes have a stainless steel shank along the cable to easily maneuver the probe head on the vessel and maintain position of the probe with a micromanipulator. As with chronic probes that have no handle, do not apply pressure or force to the probe head or reflector.
- We suggest using fine forceps to lift the vessel into the probe lumen, rather than using the reflector hook to scoop the vessel into the prove lumen.
- Keep sharp objects away from the reflector face and probe body (scalpels can chip the reflector surface and damage the epoxy probe body).

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Transit-time Perivascular Volume Flowprobes Cont.

CHOOSING A FLOWPROBE FOR SMALL VESSELS

Transonic® Nanoprobes and V-Series Probes produce repeatable, high resolution volumetric blood flow measurement data on vessels as small as 250 micron diameter. Both styles are cited in the literature for flow measurement studies in the mouse.

PS-Series Nanoprobes

- Acute and chronic use: Flowprobes may be configured for acute anesthetized studies or for chronic implantation with short cables and small connectors. The subjects can then be recovered and measurements taken while the animal is conscious over a period of days, weeks or months.
- Smaller Probe body: the Probe occupies minimal space in the surgical field and fits small anatomical spaces such as the mouse renal cavity.
- Measurements are less sensitive to vessel position within Probe lumen. The smaller rectangular lumen of Nanoprobes requires only general vessel position for proper ultrasonic illumination (Fig. 1). The vessel should fill 75% or more of the Probe lumen for best accuracy.
- Small amount of coupling gel needed to fill air space between the Probe and vessel
- Smaller measurement scale; more appropriate range for small vessel flow rates.
- Delicate construction.
- Can be difficult to place vessel within Probe lumen because the reflector is thicker than the metal V-Probe reflector.

V-Series Flowprobes

- Acute use only: suppled with stainless steel handle (non-handle versions may be custom ordered).
- Larger physical Probe size for small diameter vessel (Fig. 1); occupies more space in surgical field and requires a longer isolated vessel segment.
- Not a major issue on mouse carotid application because vessel is long and without branches.
- Size is a problem on the mouse renal artery where space is limited and the vessel has many small branches.
- Position sensitive; gives erroneous readings if used incorrectly. Vessel must be positioned in bottom of the V (Fig. 1) defined by the reflector even though the Probe lumen is much larger.
- Requires more coupling gel to fill up large air space.
- Rugged construction.
- Thin metal reflector: easier to place vessel within Probe.

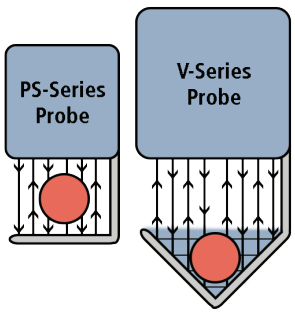


Fig. 1: The full height of the PS-Series Probe's ultrasonic window has the same flow sensitivity, so that the vessel can be positioned any-where within the Probe. Only within the triangle (shaded) portion of the V will the V-Series Probe reach its full flow sensitivity.

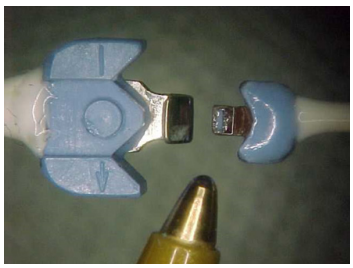


Fig. 2: V-Series and PS-Series probes next to a ballpoint pen tip for scale.

Keys to Accurate Perivascular Flow Measurements with Transit-Time Ultrasound

Importance of Acoustic Coupling for Accuracy

Highest accuracy with ultrasonic transit-time Flowprobes is achieved when the ultrasound signal is transmitted under uniform acoustic conditions. This occurs when the acoustic properties of the coupling media and tissue are stable and most closely match the acoustic properties of the liquid being measured. Since volume flow measurement with Transonic® Flowprobes is derived from a phase shift (the difference in upstream and downstream transit times) and is impacted by changes in the acoustical velocity of the ultrasonic beam, discrete sources of error from acoustical mismatch can be eliminated by observing the following guidelines.

Fig. 1: Upper graphic shows a Perivascular Flowprobe without acoustic couplant. Bottom graphic shows the same Flowprobe with acoustic couplant filling the spaces between the Probe and the vessel.

AIR

Air attenuates the Probe's ultrasound signal and effectively blocks ultrasound transmission. With large air pockets in the path of the ultrasound beam, the Flowprobe receives little or no transmitted signal and accurate flow measurements are not possible. Even small air bubbles can compromise measurement accuracy. Therefore, all spaces between the vessel and Probe must be filled with a suitable coupling agent (Fig. 1).

COUPLANT

The best acoustic couplant is Surgilube (E Fougera & Co.) because it matches the acoustic properties of blood. Media with lower acoustical velocity and impedance than blood are poor coupling agents for blood flow measurement with current ultrasonic transit time Flowprobes. These agents include saline, water, and NALCO 1181 mixed with saline. Aquasonic 100, an acoustic coupling agent used for sonography proved to be only on the borderline of acceptability for use with transit time Probes. Acoustically mismatched media cause reflections of the ultrasound at the vessel boundary, can substantially change the acoustical beam direction within the Probe, and impose uneven changes in the ultrasonic transit time. Measurements may be unstable, noisy and unpredictable in both positive and negative directions.

FAT

Fatty tissue also has a low acoustic velocity and affects the ultrasonic beam similarly. A pad of fat on the vessel wall in the acoustic pathway of the ultrasonic beam can act like a lens, reflecting or defocusing the ultrasound and altering the transit time.

TEMPERATURE

Temperature also effects the velocity of ultrasound and should be controlled for the most accurate measurements. Acoustical velocity increases with temperature increase. Transitions of the ultrasound beam from room temperature coupling agent to body temperature vessel wall and blood will alter the transit time and may exacerbate errors from other sources.

Keys to Accurate Perivascular Flow Measurements Cont.

CHOICE OF A PERIVASCULAR PROBE

Although Transonic® Flowprobes are designed for a non-constrictive fit on the vessel, the vessel/Probe fit can influence accuracy significantly. For acute applications, the vessel must fill at least 75% of the Flowprobe lumen to meet published accuracy specifications. A close or snug fit will result in the least measurement variability. A close fit lessens the amount of acoustic gel needed and minimizes its effect on the measurement.

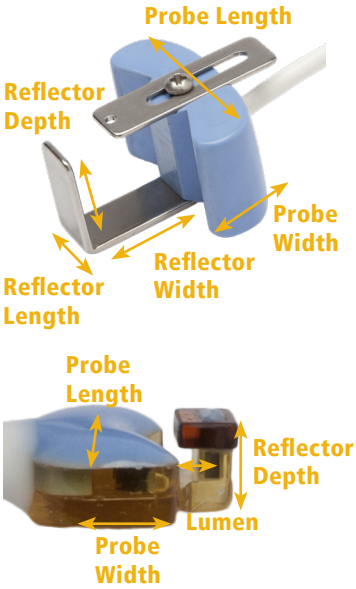
- Choose a range of Flowprobe sizes to cover variability in vessel diameter between subjects so that the 75% vessel fit rule is followed.
- Use Nanoprobes for a close fit on small vessels (< 700 microns) to maintain acoustic coupling more easily.
- Certain Flowprobes have been designed with increased sensitivity to minimize the effects of acoustic mismatch. These include V-Series Flowprobes for small vessels (<700 micron diameter). V-Series Probes are larger bodied and may be used instead of Nanoprobes as vessel length and surgical space allows being careful to follow vessel placement guidelines for V-Series Probes.

SUMMARY

Subtle phase shifts in the ultrasonic beam may be caused by inappropriate acoustic conditions during the experiment and will affect the accuracy of the measurement. Acoustically tested and approved coupling agents should be used with Transonic® Flowprobes. Fatty tissue should be carefully cleaned from the vessel where the Probe is placed. Controlling temperature in the acute experiment makes excellent physiological sense, in addition to being good acoustic practice. Transonic® Perivascular Flowprobes are calibrated for measurements of blood at 37°C and will give the most accurate readings if used within a ± 2 - 3 degree range. Gels may be warmed on a heating plate and the Probe itself should be allowed to equilibrate to this temperature for about an hour prior to use.

Transit-time Volume Flowprobes Specifications

PROBE SIZE & SERIES	PROBE BODY			REFLECTOR			HANDLE	CABLE	
	WEIGHT	LENGTH	WIDTH	LENGTH	WIDTH	DEPTH	LENGTH	LENGTH	DIAMETER
	grams	mm	mm	mm	mm	mm	cm	m	mm
0.5PS	0.09	3.2	2.3	lumen = 0.47[1]		1.0[2]	5[3]	60 cm	1.0
0.7PS	0.12	3.2	2.7	lumen = 0.70[1]		1.2[2]	5[3]	60 cm	1.0
1.5PS	0.23	4.25	3.75	lumen = 1.65[1]		2.0[2]	N/A	60 cm	1.25
0.5V	0.2	6.5	4.0	2.0	1.5	1.1	5[3]	60 cm	1.5
0.7V	0.25	7.6	3.5	2.5	1.7	1.8	5[3]	60 cm	1.5
1PR	0.2	6.5	4.0	2.0	1.5	1.1	N/A	60 cm	1.5
1.5PR	0.25	7.6	3.5	2.5	1.7	1.8	N/A	60 cm	1.5
2PS	0.3	8.7	3.3	3.3	2.0	2.5	N/A	1.0	1.5
2.5PS	0.3	8.7	3.3	3.3	3.2	2.5	N/A	1.0	1.5
3PS	1.2	9.0	5.0	3.5	4.0	3.7	N/A	1.0	2.0
4PS	1.5	13.3	6.0	3.8	5.5	4.4	N/A	1.0	2.0



1. Lumen is the ultrasonic window within the reflector. Dimensions are approximately equal.
2. Reflector depth is the external dimension for Nanoprobes.
3. Handle comes standard with indicated MA- acute Probes. MC- chronic or custom orders are available without a handle.

PROBE SIZE & SERIES	VESSEL OD		BIDIRECTIONAL FLOW OUTPUTS				ACCURACY SPECIFICATIONS[5]			ULTRASOUND FREQUENCY
	MA-ACUTE APPLICATION	MC-CHRONIC APPLICATION	RESOLUTION[1]	LOW FLOW (¼ SCALE)[2]	STANDARD SCALE[2]	MAX FLOW[3] (STD SCALE)	ZERO OFFSET[4]	ABSOLUTE ACCURACY	RELATIVE ACCURACY	
	mm	mm	ml/min	1V output in ml/min	1V output in ml/min	5V output in ml/min	ml/min	% of reading	%	MHz
0.5PS	0.3 - 0.5	0.3 - 0.48	0.03	1.5	6	30	± 0.12	± 15	± 2	14.4
0.7PS	0.5 - 0.7	0.4 - 0.7	0.05	2.5	10	50	± 0.2	± 15	± 2	9.6
1.5PS	1.2 - 1.5	1.2 - 1.5	0.075	10	40	200	± 0.8	± 15	± 2	4.8
0.5V	0.25 - 0.5	N/A	0.05	2.5	10	50	± 0.25	± 15	± 3	7.2
0.7V	0.35 - 0.7	N/A	0.075	5.0	20	100	± 0.5	± 15	± 3	4.8
1PR	0.7 - 1.2	0.7 - 1.0	0.05	5	20	100	± 0.2	± 10	± 2	7.2
1.5PR	1.2 - 1.8	1.0 - 1.5	0.075	10	40	200	± 0.4	± 10	± 2	4.8
2PS	1.5 - 2.0	1.3 - 1.8	0.1	25	100	500	± 1	± 10	± 2	3.6
2.5PS	1.8 - 2.5	1.5 - 2.4	0.1	25	100	500	± 1	± 10	± 2	3.6
3PS	2.5 - 3.7	2.4 - 3.4	0.4	50	200	1 L	± 2	± 10	± 2	3.6
4PS	3.3 - 4.4	3.0 - 4.0	0.8	100	400	2 L	± 4	± 10	± 2	2.4

1. Resolution: represents the smallest detectable change in flow, a factor in accuracy.
2. Probes operate in either low flow or normal flow scales, determined by the flow range under study. Probes measure bidirectional flow up to 5 times the selected scale setting. The scale settings calibrate the 1 volt reference signal for data collection; the linear range of the Flowmeter is equal to ± 5V. By using the low flow button, measurement sensitivity is increased by a factor of four. This linear overrange is important for the proper recording of highly pulsatile peak flows.
3. Maximum Range for each Probe reflects the highest flow rate that can be processed.
4. Zero offset on individual Probes is often lower than this value.
5. The Absolute Accuracy percentage can be raised to relative accuracy levels by in situ calibration.

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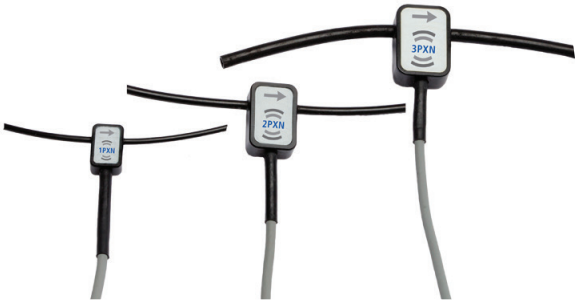
Transit-time Inline Tubing Flowsensors

PXN Inline Flowsensors splice into laboratory tubing and measure volumetric flow of blood and other fluids. The flowsensors are compatible with the TS410 Tubing Flow Module installed in a T402 or T403 Console. They offer the most flexibility for flow measurement in circuits where tubing requirements have not been formalized. The four-transducer Sensor design offers precise and accurate flow measurement for low or high flow rates, steady state or pulsatile flows. Flow resolution is scaled to Sensor size, and flow is measured accurately across the Sensor's full dynamic range with little effect from turbulence. The Sensor's smooth round flow channel is easy to clean and does not trap air bubbles that can degrade ultrasonic performance. Miniature size 1PXN - 3PXN Sensors are fabricated around flexible tubing which may be cut to length for insertion into small tubing circuits or perfusion apparati. The flexible tubing ends may be expanded to fit over larger tubing OD (see RL-31-tn). PXN Inline Sensors can be calibrated and pre-programmed for up to 4 fluid, temperature, and flow rate combinations. Ideal for flow measurement in isolated heart apparati.



SENSOR SIZE	TUBING SPECIFICATIONS				INLINE FLOWSENSOR CALIBRATION RANGES[1]			
	TUBING ID		BARB OD		LOW FLOW (1/4 SCALE)[2]		STANDARD FLOW (FULL SCALE)	
	in	mm	in	mm	LOWER LINEAR LIMIT[3,4]	MAX MEASUREMENT RANGE[5]	LOWER LINEAR LIMIT	MAX MEASUREMENT RANGE[5]
1PXN	3/64	1.2	Flexible tubing		5 ml/min	-25 to +25 ml/min	10 ml/min	-100 to +100 ml/min
2PXN	1/16	1.6			10 ml/min	-50 to +50 ml/min	20 ml/min	-200 to +200 ml/min
3PXN	3/32	2.4			25 ml/min	-125 to +125 ml/min	50 ml/min	-500 to +500 ml/min

1. To meet accuracy specifications, Flowsensors should be calibrated for the flow rate range of use.
2. Flowsensors calibrated for the low flow range should be used with the Flow Module in "1/4 Scale Mode."
3. Measurements below the Lower Linear Limit may deviate from the stated accuracy specification.
4. Custom calibration is available for average flow rates below the lower limit. This may compromise accuracy for the max measurement range.
5. Range includes zero: any flow peaks exceeding the Max Flow Value (-5 volt to +5 volt range) will be clipped.
6. Standard calibration range is up to 30 L/min; contact factory for availability of higher flow rate calibrations.

SENSOR SIZE	BIDIRECTIONAL FLOW OUTPUTS				SYSTEM ACCURACY SPECIFICATIONS[1]			PHYSICAL SPECIFICATIONS[4]				ULTRASOUND FREQUENCY
	RESOLUTION	LOW FLOW (¼ SCALE)	STANDARD FLOW SCALE	MAX FLOW (STD SCALE)	MAX ZERO OFFSET[2]	ABSOLUTE ACCURACY	LINEARITY[3]	TOTAL LENGTH W/ TUBE ENDS		CASE LENGTH W/O TUBE ENDS		
	at 10 Hz in ml/min	1V output in ml/min	1V output in ml/min	5V output in L/min	ml/min	% of reading	%	in	mm	in	mm	MHz
1PXN	± 0.02	5	20	100	± 0.4	± 8	± 2	3.9	100	0.3	8	9.6
2PXN	± 0.02	10	40	200	± 0.6	± 4	± 2	3.9	100	0.5	12	9.6
3PXN	± 0.05	25	100	500	± 1	± 4	± 2	3.9	100	0.6	14	7.2

1. Stated system accuracy specifications apply to PXN Flowsensors with TS410 Flow Modules
2. Zero offset can be eliminated by Zero Adjustment prior to measurement
3. Within specified calibration range.
4. Standard cable length is 1.85 meters.

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400-Series Quick Start Guide – Initial Setup

This is only a basic guide. Please refer to the user manual for complete operational instructions.

Transonic's 400-Series Modular Instrumentation Consoles present researchers with the opportunity to configure their flowmeter set-up to meet their specific application needs. The user can opt at purchase to have one, two or three channels of volume flow, choice between in vivo vascular flow measurement capability or flow measurement in extracorporeal tubing models, and can add a two channel pressure module to simultaneously measure flow and pressure. Each module has features that are specific to the type of measurement to therefore optimize its use and the data it will acquire. The following Reference Guide will walk you through the highlights of each module type (though you may not have each of these installed in your configuration).

Fig. 1: Front of a 400-series console (T403) with TS410 Tubing Flowmeter Module, TS420 Perivascular Flowmeter Module, SP430 Pressure Module (L to R).

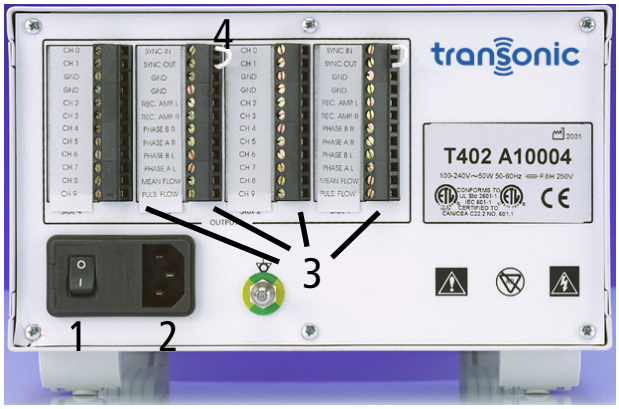

Fig. 2: 400-Series Console (T402) rear panel with power switch (1), line power access (2), secondary signal outputs (3) and synchronization access (4).

Set up the flowmeter on a table, equipment bench or cart and identify the module capabilities that you have. The 400-series Console Hardware and Data Acquisition System/Computer should be positioned within 1 to 1.5 meters of your surgical area to ensure adequate cable length to reach your experimental set-up. Note: Extension cables of various lengths are available and recommended so that your surgical area is not cramped or cluttered.

Plug in Line Power Cable (1) and proceed to the module type that is installed in your console for specific operational instructions.

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TS420 Perivascular Flow Module Quick Start Guide – Initial Setup

TS420 Perivascular Flow Module Operational Features

1. Flowprobe Connection
1A. Calibration Key Port (4-pin chronic probes only)
2. Analog out to DAQ (BNC Cables)
3. Signal Quality of Flowprobe
4. Digital Display of Volume Flow in mL/min or L/min
5. MODE Selection: MEASURE, TEST, ZERO, SCALE
6. Filter Setting: 160 Hz, 40 Hz, 10 Hz, 0.1 Hz
7. Low Flow Range Setting
8. Invert Flow Polarity Setting
9. Needle Meter corresponds to scale voltage
10. Zero Adjust Setting

SET-UP

1. Connect Flowprobe or Flowprobe Extension Cable to Probe input # 1.
   Note: Nanoprobes require use of an extension cable for adequate signal quality.
   Note: If using chronic 4-pin probes, plug flowprobe specific eprom key in port 1A.
2. Connect BNC cable to BNC output connection # 2. Connect the other end of this cable to your chosen DAQ Input Channel for FLOW.

Complete steps 1 & 2 for all installed modules (TS420; TS410; SP430)

3. Using power switch on rear of the 400-series console, turn on the system and verify that green lights are illuminated in the modules present in the console.

With steps 1, 2 and 3 completed, you can now establish a "Template File" in your chosen software platform. Template files – also known as "settings files" – are software files that have specific calibration data saved for output channels provided by the 400-series.

NOTE: Settings Files for Flow measurements have flowprobe size specific scaling, so it is important to ensure that the DAQ calibration template is scaled for the correct size flowprobe if you are using different sizes or change to the "Low Flow" scale during experiment.

In the 'Software set-up and data collection' chapter of this workbook you will find several documents on how to create template files with different software platforms.

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TS420 Quick Start Guide – Daily Use Checklist

Note: If equipment is not yet connected, reference "400-Series Quick Start Guide – Initial Setup" on page 29

1. Turn on Equipment

- Turn on the 400-Series console by switching the power button on the back panel.
- Start the Data Acquisition System (DAQ), load your software program, and select your pre-saved template file.

2. Test the signal quality of the probe

- Connect probe to TS420 meter in TEST mode (# 5).
- Immerse the probe in soft plastic beaker with degassed water or saline and move back and forth to remove small air bubbles that block signals.
- Observe Flow Module's front panel signal quality indicator (# 3) and Digital Display (# 4). The "No Sig" message will be replaced with "Good Sig" as good acoustic conduction is established with the Probe.
- The Signal Quality Indicator should be fully illuminated - all 5 bars will be lighted.
  NOTE: If the Flowprobe has less than 3 bars lit during the water/ saline test (without interruption from bubbles) do not use the Flowprobe for measurements. Contact a Transonic Representative.

3. Select the Filter Setting (# 6)

Low pass filters permit selection of the best frequency response to record accurate waveforms. The filter should be set to 160 Hz for the high heart rates of rodents.

4. Establish the Flowprobe Scale

The flow range of each Flowprobe is scaled according to Probe size. Best practices recommends that you record a two point calibration cycle at the beginning of each recording to confirm the scale settings of the DAQ file against the flowmeter setting.

- Select ZERO Mode (# 5) to output 0 volts
- Select SCALE Mode (# 5) to output 1 volt. The digital display (# 4) will indicate the value of the 1 volt scale for the flowprobe. This value must match your DAQ scale.

Under normal physiological conditions for a given vessel size, mean blood flow values are generally near or below the 1 volt normal scale value for the appropriate Probe size for the vessel. The Low flow setting (# 7) is 1/4 of the normal range to amplify exceptionally low flow signals and provide a four-fold increase in sensitivity.

NOTE: Engaging the Low Flow setting will change the Scale setting and must be also changed in your DAQ recording or recorded values will be erroneous.

NOTE: Peak flows exceeding 5 x scale (5 volts) will be clipped or flattened. You must choose the scale setting (normal or low) that will capture a complete pulsatile waveform even if the average flows are lower.

5. Adjust Zero Offset (when present)

Adjust any zero offset by pushing the recessed ZERO ADJ button (# 10) using a blunt stylus. Best practice would be to zero the flowprobe on the vessel with flow stopped or occluded.
If zeroing the prove in a container, be sure the condition is stable.
Note: Reflections on glass containers can cause drift.

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TS420 Quick Start Guide – Daily Use Checklist Cont.

6. Begin Measurements

- Put the flow meter in MEA Mode (# 5) and place the probe around the vessel of interest assuring that there is good acoustic coupling (all 5 signal bars should light up).

Note: Air blocking the ultrasound signal can be replaced by acoustic gel or warm saline to assure good coupling. Best practice is to use SurgiLube Gel.

- Transonic Flowprobes are bidirectional and may be applied to the vessel in the best direction dictated by the anatomy. If measured flow is negative; reverse polarity of the probe by engaging the Invert button (# 8).

7. Start Collecting Data

After confirming scale settings above, begin recording. Cycle through the 2-point reference calibration signals and start collecting data.

SP430 Pressure Amp Module Quick Start Guide – Set-up & Daily Use Checklist

SP430 Pressure Amp Module Operational Features

1. Pressure Sensor Inputs (HDMI Cable)
2. Analog Outputs to DAQ (BNC Cables)
3. Operational Controls: MEA – Measure
4. Operational Controls: 0 mmHG Reference Calibration
5. Operational Controls: 100 mmHG Reference Calibration
6. Balance Controls

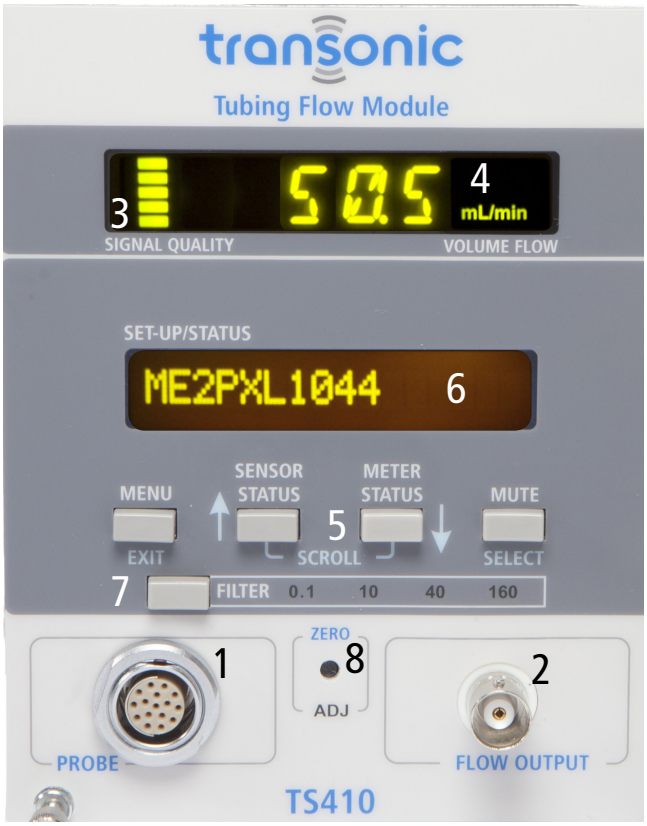
SET-UP

1. Connect Pressure Sensor(s) to HDMI Cable(s) and connect to SP430 input(s) # 1.
2. Connect BNC cable(s) to BNC output(s) connection # 2.
3. Connect the cable(s) to your chosen DAQ Input Channel(s) for PRESSURE.

DAILY USE CHECKLIST FOR SP430

Follow the set up and daily use steps for catheter hydration, use and care as for the SP200 and pressure catheters on page 12.

TS410 Tubing Flow Module Quick Start Guide – Initial Setup



TS410 Tubing Flow Module Operational Features

1. Flowsensor Connection
2. Analog out to DAQ (BNC Cable Connection)
3. Signal Quality of Flowsensor
4. LED Digital Display of Volume Flow in mL/min or L/min
5. Programmed MENU Modes
    a. Sensor Status
    b. Meter Status
    c. Sensor Calibration Settings
    d. Meter Settings (Scale Settings, Alarms)
6. LCD Message Display
7. Filter Setting: 0.1 Hz, 10 Hz, 40 Hz, 160 Hz
8. Zero Adjust Setting

Tubing flowmeters are most commonly used in mock circulation systems, such as Langendorff, working heart and other organ studies.

SET UP

1. Connect Flowsensor to sensor input # 1.
2. Connect BNC cable to BNC output connection # 2
3. Connect the other end of this cable to your chosen DAQ Input Channel for FLOW.

Transonic Tubing Flowsensors are calibrated for specific conditions (type of liquid, temperature of the liquid, flow range expected and -in case of clamp-on sensor- tubing material and size). These parameters are programmed in the sensor connector and are selectable. Up to 4 sets may be defined at purchase. Sensor calibration parameters must match your set up.

TS410 Quick Start Guide – Daily Use Checklist

1. Install the flowsensor in your tubing circuit, or isolated organ apparatus

The Flowsensor should be applied on straight tubing segments, about 10X the ID from side branches to produce measurements within its accuracy specifications.

For Inline Flowsensors: Mate the sensor tubing ends with your tubing. Mounting the flowsensor vertically so flow direction rises will help to minimize air bubbles in the sensor.

For Clamp-on Tubing Flowsensors: Apply a thin layer of petroleum jelly, or silicone grease over the tubing surface to enable ultrasonic transmission through the tube and insert the tubing into the sensor slot and close lid.

2. Press Sensor Status button (# 5)

The LCD (# 6) will indicate tubing / fluid / temperature / and calibrated flow rate setting. These settings need to match the current application. If not, select the appropriate calibration setting by entering the user interface MENU.

For full explanation of the menu navigation see "TS410 Quick Start Guide – Program Menu" on page 36 or refer to the 400-series manual.

3. Prime the circuit and check Signal Quality (# 3)

Allow 5 minutes for the Sensor to equilibrate on the tubing. Good signal is indicated by 4-5 bars. Lower signal bars may indicate air in the tubing, mismatched calibration parameters (wrong tubing, different temperature, different fluid), or possibly unclean or degraded sensor surfaces. Lower signal will still allow you to make measurements, though accuracy may be compromised.

4. Record Calibration Reference Signals in DAQ: Zero and 1 volt Scale

Enter the Menu (# 5) [Meter Controls] to [Calibrate Scale] and select [0 Volt Scale] then select [1 Volt Scale] to output the reference signals to your DAQ. Reference calibration values will be displayed on the digital LED display (# 4).

Note: Scale values are dependent on Flowsensor size and scale selection. Like the TS420, there is a ¼ scale flowmeter setting in the program which can be selected for low flow applications.

5. Stop Flow and Zero the Flowsensor

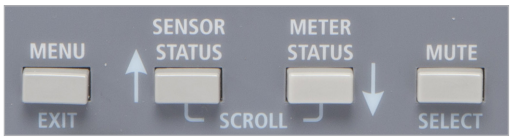
Tubing sensors have some offset at zero flow caused by the tubing, Once the sensor has equilibrated and flow has stabilized, use the recessed zero adjust button (# 8) to null the offset at zero flow.

6. Begin Measurements and Data Collection

You are now ready to begin taking measurements with the TS410.

TS410 Quick Start Guide – Program Menu

Press **[Menu]** to enter the program, this makes the blue button labels active. Use arrows ↑↓ to **[Scroll]** through menus and to set values for alarms and gain (pressing and holding scrolls faster). Press **[Select]** to enter menus or toggle settings on and off. A star (*) indicates that a selection is made/active. Return to Measure mode at any point using the **[Exit]** button.



Cleaning & Sterilization of Transonic® Flowprobes

Device	All Transonic® Reusable Research Perivascular Flowprobes
Warnings	Flowprobes are delicate precision instruments and should be handled carefully at all times. It is critical that the Probe connector be completely dry before use. Air dry or carefully wipe with disposable cloth/paper.
Limitations and restrictions on reprocessing	Connectors for acute use are not sealed and should not be soaked. Connectors for chronic use may be momentarily immersed, then rinsed to remove debris prior to sterilization. Repeated processing has minimal effect on instruments. End of life is normally determined by wear and damage due to use. **NOTE:** Care must be taken when scrubbing the softer materials near the probe head to prevent damage to the silicone.
Preparations at the point of use	Remove excess debris with disposable cloth/paper. Wipe and/or rinse with water to remove excess bio-materials.
Containment and transportation	No particular requirements. **NOTE:** It is recommended that instruments are processed as soon as is reasonably practical following use. Dried-on materials are more difficult to remove.
Cleaning preparations	Flowprobes with sliding cover should be disassembled for a thorough cleaning.
Cleaning solutions	Alkaline, Neutral or Enzymatic. Use only those cleaning agents approved by your governing regulatory agency. Use all cleaning agents according to the manufacturer's directions.
Manual cleaning	**[1]** Rinse excess soil from instrument (temp <30°C, 86°F). **[2]** Using detergent (e.g. Steris Prolystica 2X concentrate Neutral Detergent) and soft brush remove any visible foreign material on all probe and handle surfaces for 3 to 5 minutes. Soaking or immersion in detergent during brushing is allowed. **NOTE:** Excess or aggressive scrubbing of the probe neck can damage the silicone, particularly where it is sealed to the handle. **NOTE:** Connector surfaces may be wiped clean with solutions, but take care not to damage connector pins. If solution gets on pins, carefully wipe them dry as soon as possible. **[3]** Rinse with tap water. **[4]** Visually inspect for cleanliness and repeat cleaning if necessary.
Automatic cleaning	Only use cleaning solutions which have been approved for use with an automatic washer (e.g. Steris Prolystica 2X Concentrate Alkaline Detergent). **[1]** Detergent wash minimum of 2 minutes in hot tap water **[2]** Rinse minimum of 2 minutes at 70°C **[3]** Dry minimum of 15 minutes at 80 °C **[4]** Visually inspect for cleanliness and repeat cleaning if necessary. **NOTE:** Do not exceed 90°C unless the device has the autoclave label on the connector.
Disinfection	After cleaning, all Probes must be sterilized. Additional disinfection is not required and may ultimately damage the Probe. Use only those disinfecting solutions approved by your governing regulatory agency. When disinfection is performed, follow the manufacturer's instructions applicable to the disinfection solution.
Packaging for sterilization	A polyethylene/tyvek pouch sized per the table above may be used provided it is approved by the appropriate regulatory agency for use with the desired sterilization method. Ensure that the pack is large enough to contain the instrument without stressing the seals. Use a pouch that is validated for the specified sterilization cycle. Use approved sterilization wrap to cover instrument tray for Sterrad sterilization according to manufacturer's instructions.

Cleaning & Sterilization of Transonic® Flowprobes

<table>
<tr><td rowspan="2">Sterilization</td><td>STERRAD

STERRAD 100
STERRAD 100s: Short cycle
STERRAD 100NX: Standard cycle
STERRAD NX: Standard cycle
STERRAD 200: Short cycle

Follow the instructions for use provided with the STERRAD machine for proper sterilization processing.</td><td>Ethylene Oxide (ETO)

PRECONDITIONING
Humidity: 55-75% RH
Temp: 38-50°C (100-122°F)
Time: 12 hours

EXPOSURE (600±50 mg/L, 3 hours)
Vacuum: 0.8 ± 0.5 "HgA
Sterilant gas: 100% EO
Humidity: 2.4 ± 0.5 "HgA
Temp: 49-54°C (120-130°F)
Time: 3-3.5 hours

POST EXPOSURE
Vacuum: 1.0 ± 0.5 "HgA

AERATION
Temp: 43-55°C (109-131°F)
Time: 12 hours</td></tr>
<tr><td>STERIS

V-PRO MAX
Non-lumen, cycle time = 28 min
Lumen, cycle time = 60 min

V-PRO 1 PLUS
Non-lumen, cycle time = 28 min
Lumen, cycle time = 60 min

V-PRO 60
Non-lumen, cycle time = 28 min
Lumen, cycle time = 60 min</td><td>STEAM (AUTOCLAVE)
Not available for PXN, PXL, PS, PR or PAU series
Only reusable Flowprobes with this symbol on the connector can be autoclaved.

GRAVITY DISPLACEMENT STEAM STERILIZATION:
132°C for 15 minutes with 30 minutes dry time
135°C for 10 minutes with 30 minutes dry time

DYNAMIC AIR REMOVAL STEAM STERILIZATION:
132°C for 4 minutes with 20 minutes dry time
134°C for 3 minutes with 20 minutes dry time</td></tr>
<tr><td>Inspection, maintenance and testing</td><td colspan="2">Inspect each Perivascular Probe for:
• A bent reflector (the reflector should be at a right angle to the Probe body).
• Cracks or chips in the plastic Probe body.
• Nicks in the Probe cable (if nicks are observed, do not reuse).
• Damage to the silicone seal (if integrity of the silicone is compromised, do not reuse).
Consult the Flowmeter's Operator's Manual for testing instructions.</td></tr>
<tr><td>Storage</td><td colspan="2">The probe is ready for use after sterilization is complete. There are no additional storage requirements.</td></tr>
</table>

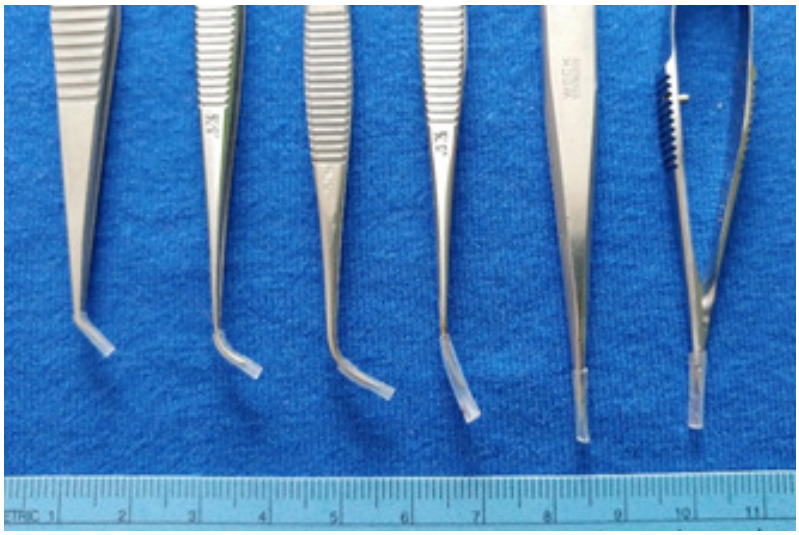
The instructions provided above have been validated by the manufacturer for preparing a device for re-use. It remains the responsibility of the reprocessor to ensure that the reprocessing as actually performed using equipment, materials and personnel in the reprocessing facility to achieve the desired result. Your reprocessing procedure should comply with local regulations.

Surgical Instruments for Acute Rodent Procedures

To assure that the data collected with Transonic and Transonic Scisense equipment is the best quality data possible, one must start by performing careful surgery. This means not only having good microsurgical skills and knowledge of the anatomy of your animal, but also good surgical tools.

CLEANING AND TAKING CARE OF SURGICAL TOOLS

Surgical tools need to be chosen with care before you start your surgery. Excess instrumentation in your surgical field will complicate your procedure when working with the microscope, so only have the equipment on the table necessary to perform a given surgery.

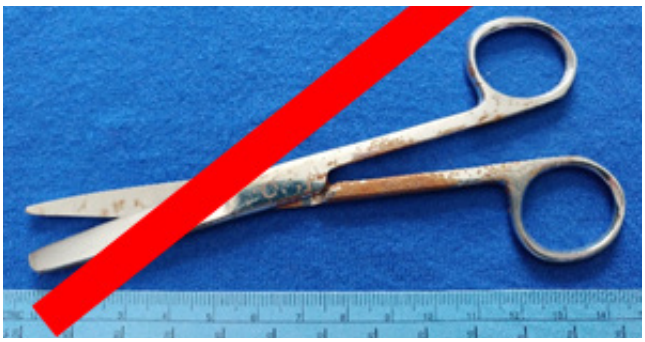


Grabbing and cutting ends of micro instruments are protected by polyethylene tubing.

For rodent work, the most valuable instruments are the micro instruments. Taking good care of these instruments during usage and storage, preventing forceps' tips to misalign and dissecting scissors from becoming blunt, assures that they will serve you well during surgery. When working ends of micro instruments are damaged, inserting pressure catheter might for instance be challenging. On the picture right, grabbing and cutting ends of micro instruments are protected by polyethylene tubing. This is a simple but effective step, to ensure that stored equipment is protected from hitting each other and other hard surfaces. Before starting surgery, check the instruments you are going to use. Good preparation makes a difference during surgery.

Soft bristle toothbrush works well for cleaning.

Soft brushes or an ultrasonic bath are the best methods to clean your surgical instruments. Use low foaming mild cleaning solutions to hand clean them or a solution compatible with ultrasonic bath instrument cleaner e.g. Citranox, Alconox or Liquinox. If you decide to use an ultrasonic bath, clean a few micro instruments at once to avoid the instruments damaging each other while in the bath. Keep locks and ratchets open during cleaning for better penetration of cleaning solutions to remove clotted blood and other debris. Clean your crude surgical instruments first followed by micro instruments. Crude instruments should not be in direct contact with micro instruments during cleaning, handling, drying or storage.



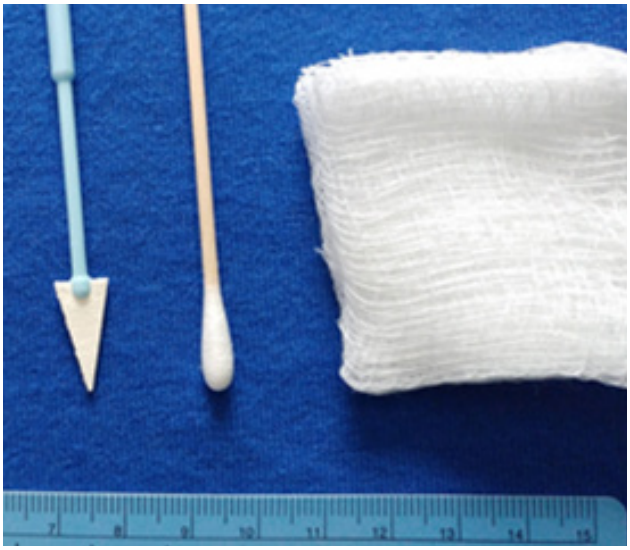
Do not use rusty instruments.

For acute experiments, sterilization is not absolutely necessary. However, sepsis can start to develop after 2 hours. If your protocol exceeds this time frame, it is required to sterilize your instruments. Check the best suitable sterilization method; dry autoclaving, cold sterilization, irradiation or other.

Surgical instruments are made from stainless steel. Depending on the quality of your set, instruments discolor over time. Before surgery please assess whether discoloration is stain or corrosion. Stains can be erased by a pencil eraser. If it is rust, it is time to discard your instrument. Rusty instruments won't serve you well during surgery.

Surgical Instruments for Acute Rodent Procedures Cont.

During vessel opening while inserting a Transonic Scisense pressure catheter, bleeding might occur. Select the appropriate tools to stop bleeding based on the size and location of the bleed.



Several suggestions to stop bleeding. From right to left are depicted 2" by 2" gauze sponges, 6-inch (15.2cm) cotton tipped applicator and lastly cellulose Ultracell Eye spear. Cellulose Eye spears are used for smaller bleeding from e.g. vascular opening of mouse femoral artery, cotton tipped applicator can be used to stop bleeding from rat aorta.

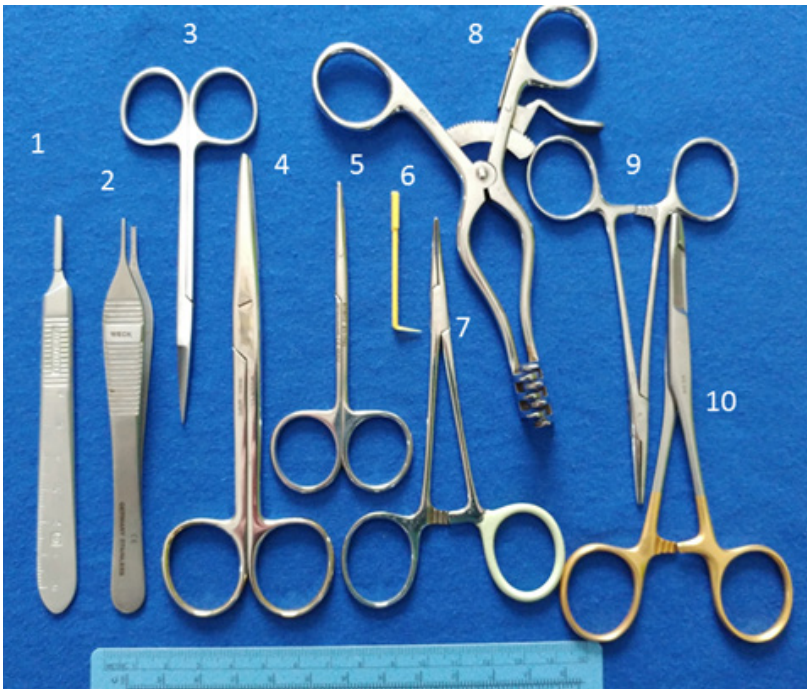
SELECTING INSTRUMENTS FOR ACUTE VASCULAR SURGERY

Selecting your instruments is a task that has to be performed in advance and not on the day of surgery. And whether you like to do all your surgical steps using a microscope, or only vessel isolation and smaller procedures in mice, it is best to practice using a microscope before performing your experimental surgery as it requires training to perform surgery under the microscope.

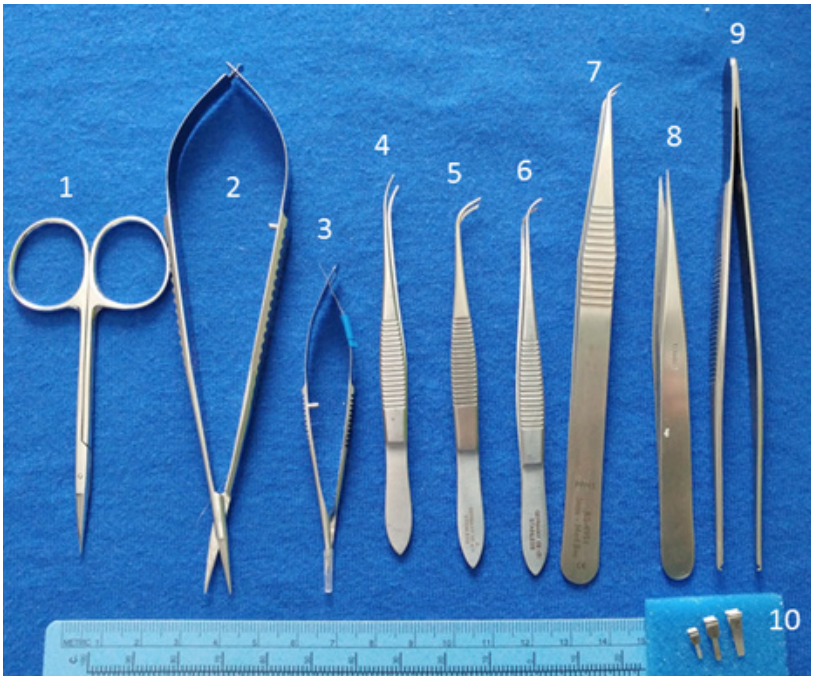
When selecting your tools, you should think about the surgical steps that have to be performed to gain access and cannulate vasculature in the area of interest. There are specific tools designed to hold skin, to divide skin and to keep it retracted.

RAT SURGICAL INSTRUMENTS

DESCRIPTION	TASK(S)
1. Bard-Parker® Surgical Blade Handle Size 3	With blade inserted; tissue separation
2. Thumb forceps Adson (1 x 2 Teeth, 1.4 mm tip, 4-3/4" (12 cm)	Hold skin
3. Fine scissors, sharp-sharp tip, cutting edge 23mm, 11.5 length	Cleaning adventitia around vessel
4. Mayo Dissecting Scissors, Straight, 5-3/4" (14.5 cm)	Divide skin
5. Micro Dissecting Scissors; Straight; Blunt; 22mm Blade Length; 4" Overall Length	Blunt dissection, cleaning around vessel
6. Catheter introducer	Catheter insertion
7. Hartman Mosquito Hemostats Curved. Overall length 5 inch (12.5cm)	Blunt dissection, cleaning around vessel
8. Adson self-retaining retractor 4x3 Blunt Prongs (14cm)	Retract skin
9. Straight Mosquito Hemostat (12 cm)	Blunt dissection, passing suture around vessel and tying surgical knots
10. Straight Olsen-Hegar Needle Holders with Suture Cutters	Tying surgical knots



Surgical Instruments for Acute Rodent Procedures Cont.



MOUSE SURGICAL INSTRUMENTS

DESCRIPTION	TASK(S)
1. Iris Scissors blade 15mm, length 9cm	Dissect skin
2. Straight Scissors S&T SAS-15 R8 15cm	Cut vessel for insertion
3. Vannas Spring Scissors Straight 2.5mm Cutting Edge	Cut vessel for insertion
4. Extra Fine Graefe Forceps, slight curve tip serrated, length 10cm	Cleaning vessel, passing suture around vessel and tying knots
5. Extra Fine Graefe Forceps, curved, serrated, length 10cm	Cleaning vessel, passing suture around vessel and tying knots
6. Extra Fine Graefe Forceps, curved, serrated, length 10cm	Cleaning vessel, passing suture around vessel
7. Dumont Jewelers' forceps, 45 Degree Angle,13.5cm	Cleaning vessel, passing suture around vessel and tying knots
8. Micro Dissecting Jeweler forceps, length 11 cm	Cleaning vessel, passing suture around vessel and tying knots
9. Micro clamp applying Forceps, S&T CAF-4, length 14cm	Temporarily stop blood flow
10. Three microsurgical clamps, vein and arterial, S&T	Temporarily stop blood flow

Additionally, a valuable tool in the surgical toolbox when installing Transonic's transit time flow probes are retractors, particularly in retraction of ribs where modified Goldstein Lacrimal sac retractor is used for rib retraction in a mouse ascending aorta acute blood flow measurement on page 96.

When it comes to these tools, you can make your own or you can rely on commercially available ones.

List of Retractors sold by:

RWD Life Science Inc. (http://www.rwdstco.com)

DBIO GmbH (https://dbio.eu/retractors.html)

Colibri Eye Specula-1.5cm spread, 4cm,

3x3 Teeth Retractors-Blunt, 4.5cm

ALM 4x4 Teeth Retractors-Blunt, 7cm

Mini Side Retractor

Goldstein Slide Retractor

WPI (https://www.wpiinc.com/surgical-instruments/surgical-accessories/retractors/wire)

Mouse Wire retractors 5cm

Barraquer retractors 4 cm

KD mouse eyelid retractors

Applications and Surgical Protocols

Now that all preparations are done, we are ready to discuss research applications that benefit from pressure and blood flow measurements. For each application we will explain how to perform the surgery or build the experimental set-up. This is done in a detailed protocol that will take you through the procedures step by step. Where we have data available, we will show you example data, so you know what you can expect.

Animal Research Guiding Principles

TRANSONIC UPHOLDS STANDARDS FOR CONDUCTING RESEARCH WITH ANIMALS

Transonic understands that animal research is a privilege requiring integrity and professionalism. Improper research practices can put animal subjects at risk, waste valuable resources, compromise careers, and delay development of new devices and technologies. Accountability and ethical behavior are parts of what we adhere to when conducting research at different institutions. Furthermore, all Transonic employees participating in animal research, product testing, or device demonstrations strive to understand all ethical principles of animal welfare and are following all federal regulations, guidelines, and local policies regulating animal research activities. We believe following regulations upholds the public's trust in the integrity of research, testing and product development in our organization.

Animal testing regulations are laws or guidelines that permit and oversee the use of non-human animals for scientific experimentation. These regulations promote high-quality science while ensuring maximum ethical treatment of animals in experimentation. Extensive evidence shows a direct relationship between science quality and animal welfare. There is a very delicate and crucial balance between scientific advances and minimum distress on animals. This balance can vary among countries, but most governments aim to control the number of times individual animals may be used; the overall numbers used; and the degree of distress that may be inflicted.

While performing research on animals, Transonic obeys laws and regulations outlined by the US, federal agencies and adhering to the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (OSTP 1985) (1). The following summary could help illustrate the similarities and differences when comparing the US versus different countries guidelines.

OUR GUIDANCE IN ANIMAL BIOMEDICAL RESEARCH

Guidance comes from history: Animal Biomedical research is based on historical milestones regarding the treatment of animals in the framework of biomedical research was published by Marshall Hall in 1831 (2). He recognized that animal experimentation was often accompanied by pain and suffering and outlined five principles that should govern the use of animals in experimentation:

1. Alternative approaches are not available, animal can be observed to obtain necessary information
2. The experiment has a clearly defined and obtainable objective
3. The work is not unduly repetitious
4. The researchers are committed to minimizing pain and suffering (use less-sentient animals)
5. The results of the studies are published in a clear and concise manner diminishing need for repetition

Later, in 1959 William Russell and Rex Burch published The Principles of Humane Experimental Technique (3). This important contribution described important ethical rules in animal research. Russell and Burch describe how pain or distress in animals could be diminished or removed through Reduction, Replacement, Refinement: the “3Rs.”

Animal Research Guiding Principles Cont.

Reduction, Replacement, Refinement: The "3Rs"

REDUCTION

Reducing the number of animals used to the minimum number to obtain reliable information to the desired level and precision

1. Employing statistical analysis to determine the appropriate sample size based on the variances of the expected data (how to best ensure proper sample size to obtain meaningful data set)
2. Using an initial pilot study with just a few animals to assess sample variances
3. Using animal or tissue-sharing programs
4. Balancing the number of animals used against the potential harm from performing multiple experiments or procedures in a single animal situation where the variances are unknown

REPLACEMENT

Substitution of a lower species for a higher one. Some examples include:

1. Replace test animals with non-animal alternative such as using computer simulations
2. Replace test animals with test animals from a less-sentient species such as fish, frogs, or mice in research instead of dogs or non-human primates. This approach is ethical only if replacement model used is appropriate for the question being studied

REFINEMENT

Refinement refers to an effort to minimize the occurrence or severity of painful procedures that have been deemed to be necessary for a study. Some examples of refinement include:

1. Using less painful or stressful research procedures
2. Using new more effective analgesics or anesthetics or more effective schedules or routes of administration.

REFERENCES

(1) Office of Science and Technology Policy (OSTP). 1985. "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training." Federal Register 50(97). Accessed June 7, 2015.

(2) Hall, Marshall. 1856. "Of the Principles of Investigation in Physiology." Lancet 1:393-4.

(3) Russell, William Moy Stratton, and Rex Leonard Burch. 1959. The Principles of Humane Experimental Technique. London: Methuen. Accessed June 7, 2015.

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THE MEASURE OF BETTER RESULTS.

Rodent Anesthesia Guidelines

There is no single best choice for anesthetic agents as procedure, parameters of interest, and animal type all impact anesthesia choice. Always check what is currently available and allowed with your Institutional Animal Care & Animal Use Committee and make sure that the anesthetic agent is balanced with proper analgesics. It is important to note that the availability of anesthetic agents changes and is dependent on your institution and country.

Below are several points of interest to consider that will help you make the correct choice within your research setting. During acute experiments, anesthetic agents have a significant influence on your measurement results, as mainly all agents influence heart function and therefore blood pressure and flow. Be aware of this when you compare your data with published results, especially when type and dose of anesthesia is not mentioned in a publication.

CONSIDERATIONS RELATED TO THE PROCEDURE

- Type of procedure
- Projected length of the procedure
- Amount and type of pain/distress anticipated
- Study goals (are important parameters influenced by certain drugs?)
- Survival or terminal study (agents associated with prolonged recovery or delayed effects may be approved for terminal studies while deemed inappropriate for survival procedures)
- Acute or chronic study

CONSIDERATIONS RELATED TO THE ANIMAL

- Species and strain
- General condition and underlying health problems
- Age
- Sex
- Weight
- Previous Drug Exposure
- Nutritional Status
- Time of day as related to circadian rhythm
- Ability to maintain body temperature (preventing hypothermia due to heat loss)
- Numbers of animals to be anesthetized simultaneously

Note: There can be remarkable variation in response to anesthesia. Investigators should monitor anesthesia closely in each animal and make appropriate modifications in the anesthetic regimen when necessary.

CONSIDERATIONS RELATED TO THE DRUG(S) USED

- Drug safety and ease of use
- Appropriateness for the procedure including administration method
- Appropriateness for the animal
- Side effects
- Equipment and training required for safe use
- Previous experience using the agent(s)
- Cost and status as controlled or uncontrolled drug

SUMMARY: ANESTHETIC AGENTS SHOULD

- Provide an appropriate depth and length of anesthesia and analgesia without affecting important study parameters
- Be appropriate for the animal given its species, medical history and physical condition
- Have minimal side effects
- Be safe for both the animal and the personnel administering anesthesia

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Rodent Anesthesia Guidelines Cont.

Cardiovascular Effects of Anesthetics

As mentioned earlier, many common anesthetics have a significant effect on cardiovascular measurements and can obscure or confound study results; sometimes over a longer period of time than anticipated. It is therefore necessary to choose an anesthesia protocol with care. For the purpose of cardiac experimental procedures general anesthesia is recommended, however dissociative anesthetics in combination with a sedative agent may be used as well.

During the experimental procedure, management of anesthesia has to be catered to any underlying or experimentally caused cardiovascular disorder. For example, experimentally induced aortic stenosis (trans-aortic banding or constriction) requires anesthesia which avoids systemic vasodilation and tachycardia while preserving sinus rhythm such as a synthetic narcotic based anesthesia.

INHALED (HALOGENATED ETHER) ANESTHETICS

It is known that inhaled anesthetics may cause circulatory depression at concentrations required to produce general anesthesia. In addition, each individual inhalation anesthetic has selective dose-dependent effects on cardiovascular function (sympathetic reflexes, intravascular volume status, vascular smooth muscle tone, myocyte contraction and relaxation, acid-base status etc.). For this reason, circulatory interactions of inhaled anesthetics might limit the anesthetic dose. Consequently, some laboratories combine inhaled anesthetics with sedatives or hypnotics to produce the necessary general anesthesia. Others empirically developed state of the art mono-anesthetic protocols using minimum amount of inhalation anesthetics to mimic close to fully-conscious state while collecting data.

Drop of blood pressure (BP) caused by inhalation anesthetics is a direct result of dose-dependent vasodilation accompanied by an afterload reduction and depression of myocardial contractility and an indirect result of attenuation of sympathetic nervous system. Decrease in BP during Isoflurane induced general anesthesia is so predictable that some laboratories often use this as a sign for assessing the depth of anesthesia.

Halogenated anesthetics decrease global LV systolic function at any given LV loading condition or at any given degree of underlying sympathetic tone. Experimental studies suggest that these agents cause minimal changes in LV diastolic compliance but impair LV diastolic relaxation in a dose-dependent manner. These agents have minimal direct effects on LV preload, but rather EDP may increase during anesthesia because of impaired diastolic filling and decreased cardiac output (CO).

The administration of inhaled anesthetics to experimental animals with cardiovascular diseases has some advantages. Most inhaled anesthetics are myocardial depressants with negative inotropic properties which decrease contractility and thus decrease myocardial oxygen demand. Arterial vasodilation combined with preserved coronary perfusion maintains oxygen delivery to the heart. Adequate oxygen delivery combined with a decreased demand for oxygen creates a more favorable myocardial oxygen balance in hearts with coronary insufficiency. Additionally, the vasodilating and antihypertensive actions of inhaled anesthetics effectively control an increase in BP in response to surgical pain.

Inhalation anesthetics have a proportionally greater negative inotropic effect on diseased myocardium compared with normal myocardium. In the case of an experimentally induced septic shock by injection of LPS or cecal puncture, profound ventricular dysfunction may not tolerate the cardiovascular depressant effects of inhaled anesthetics given in concentrations that are needed to produce the anesthesia. The pro-thrombotic side effect of sepsis causes decreased coronary perfusion pressure which prevents adequate oxygen extraction via Fick's principle. In this case cardiac oxygen demand excesses the rate of consumptions (MVO2) causing a negative oxygen balance which further depresses cardiac function.

Rodent Anesthesia Guidelines Cont.

RODENT ANESTHESIA BREATHING CIRCUITRY

Open System is the traditional method of dipping ether or chloroform on gauze, later modernized by the Schimmelbusch mask and used until about 1950.

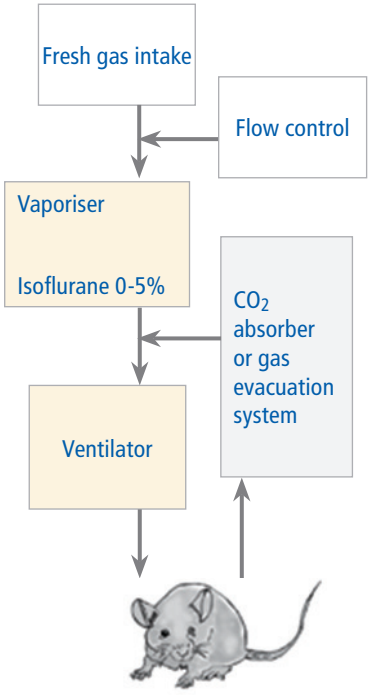
Semi-open System is commonly used today and includes all the Mapleson systems. This is typically used for animal anesthesia induction, usually a single branched system that uses a valve to control the pressure of the gas, and allows for waste gas to leave the system. This system can be further characterized by high fresh gas inflow in order to stop re-breathing of expired CO_2.

Semi-closed and Closed Systems use a CO_2 absorbent and thus gases are re-circulated; the classification (semi-open vs closed) is defined by the amount of fresh gas flow. These systems are mainly used for maintenance of anesthesia following induction. Additionally, they can be used for anesthesia induction, but this is a slower process than using a semi-open system.

Expired gases from the animal pass through a container in the breathing system which contains a CO_2 absorbent to remove CO_2 from the expired gases. This method requires a high level of animal monitoring, especially levels of inspired and expired CO_2 and the anesthetic agent. This absorbent, by an exothermic chemical reaction removes the CO_2, thus allowing an animal's expired gases to be re-breathed. Because of this exothermic chemical reaction, some warmth and humidity is added to the inspired gases. In this setting, the animal's expired gases are recirculated, allowing for a reduced inflow rate of additional fresh gas.

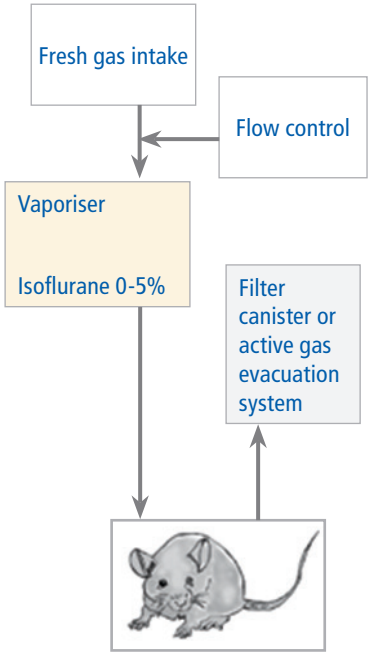
Breathing system components:

1. Fresh gas intake (O_2, medicinal air etc.)
2. Adjustable pressure and/or volume limiting valve
3. Connection to animal (ventilator)
4. Waste gas connection tubing or anesthesia gas absorber



Semi-closed system

Schema of Isoflurane inhalation semi-closed or closed system for rodent maintenance anesthesia. Unidirectional valves permit pressure driven flow through the vaporizer into the inspiratory limb of circle system. Exhaled gases are routed into expiratory limb and recirculated through use of CO_2 absorber. A bidirectional valve positioned in the expiratory limb permits gases to be evacuated if needed (e.g. high pressure develops).



Semi-open system

Schema of Isoflurane inhalation semi-open circuit (gases are not recirculated). Unidirectional valves permit pressure driven flow through the vaporizer to the Anesthetizing Box; exhaled gases are routed into Filter canister (removal of excess of Halogenated gases) or into active gas evacuation system.

Rodent Anesthesia Guidelines Cont.

SEDATIVES-HYPNOTICS

This group of anesthetics include barbiturates, benzodiazepines, etomidate, propofol and ketamine. They are used for pre-surgical sedation, producing immediate loss of consciousness, to supplement the actions of the inhaled anesthetics, and to provide sedation in the immediate postoperative period. The circulatory effects of individual agents are an important consideration for subjects with CV disease. The sedative-hypnotics have direct effects on cardiac contractility and vascular tone in addition to indirect effects on autonomic tone.

Barbiturates (e.g. sodium pentobarbital, thiopental and methohexital) are anxiolytics, hypnotics, anticonvulsants and weak analgesics with negative inotropic effects. They produce dose-dependent decrease in dP/dt and the force-velocity relationship of ventricular muscle. Induction of general anesthesia with barbiturates is associated with a decrease in blood pressure (BP), heart rate (HR) and cardiac output (CO).

In comparison with barbiturates, propofol appears to cause less myocardial depression. Mean arterial pressure (MAP) decrease after propofol is attributed primarily to both arterial and venous dilatation. Propofol is well suited for continuous i.v. infusion for sedation because it has a short duration of action and can be titrated to effect. Propofol is usually combined with opioids (Fentanyl, Sufentanyl etc.) for its lack of analgesia.

Etomidate and ketamine are administrated for rapid induction of general anesthesia in experimental animals with pre-existing hemodynamic compromise because they generally cause little or no change in circulatory parameters. Etomidate has virtually no effect on myocardial contractility even in diseased ventricular muscle. For its endocrine and neuroendocrine non-anesthetic interferences it is limited to short-term use as an i.v. induction agent.

Ketamine often increases HR and BP and causes bronchodilation because of its sympathomimetic properties. Ketamine has other beneficial effects including analgesia, anesthesia, and direct negative inotropic and vasodilatation effects

NARCOTICS (OPIOID) ANESTHETICS

Narcotic-based anesthetics offer the advantages of profound analgesia, attenuation of sympathetically mediated cardiovascular reflexes in response to pain, and have virtually no direct effects on myocardial contractility. Even though narcotics have little direct action on the heart, they may cause profound hemodynamic changes indirectly by attenuating sympathetic nervous tone while decreasing serum catecholamine levels, which may cause indirect cardiac depression.

In addition, other inconveniences encountered with narcotic-based anesthetics include difficulty estimating required dose, predicting the duration of postoperative narcotic-induced respiratory depression, and ensuring hypnosis during operation. Rapid administration of narcotics (Fentanyl) is also associated with muscle rigidity of the thoracic and abdominal musculature that may impede the ability to ventilate the patient immediately after the induction of general anesthesia.

Development of short-acting narcotic anesthetics may improve the ability to control anesthetic depth without prolonging recovery time. Ultra-short-acting narcotics (Remifentanyl) may have a unique niche in cardiac anesthesia because their effect is terminated immediately on stopping the drug infusion due to rapid *in vivo* ester hydrolysis.

ADVANTAGES OF INHALATION ANESTHESIA AS COMPARED WITH INJECTABLE ANESTHETICS

- Easily controllable cardiovascular depression
- Reduced impact on liver functions
- Reduced impact on kidney functions
- Encourages rapid recovery
- Allows superb control while on anesthesia
- Easy maintenance of surgical anesthetic depth
- Dose and volume can be easily adjusted
- Less stress on subject as compared to injections
- More predictable pharmacokinetics

Rodent Anesthesia Guidelines Cont.

CATEGORY	AGENT	SPECIES & DOSE (MG/KG)	ROUTE*	HEMODYNAMIC EFFECTS*	PMID CITATION
Anesthetics - Injectable	Alphaxolone (Alfaxan)	Mice: 15	IV	increased HR, decreased MAP	17319964
	Alphaxolone/Alphadolone (Saffan)	Rats: 18/6	IP	vasodilation	11098097, 11575348
	Chloral hydrate	Rats: 300 - 400	IP	minimal cardiopulmonary depression	8355479
	Alpha-chloralose[1]	Rats: 50-55	IP	minimal cardiopulmonary depression	19003937
	Fentanyl/Droperidol (Innovar-Vet)	Mice: 0.078/3.9	IM	vasodilation	15288130
	Fentanyl/Medetomidine	Rats: 0.3/0.3	IP	decrease HR, SV & CO, cardiorespiratory depression	22561119
	Propofol/Fentanyl/Medetomidine	Mice: 75/0.2/1 Rats: 100/0.1/0.1	IP	Vasodilation, cardiorespiratory depression	19001064, 20819392
	Propofol/Remifentanyl	Mice: 50-200/0.2-1	IP	Vasodilation, cardiorespiratory depression	17640460
	Ketamine	80- 200	IM	good HR & BP	18172330
	Ketamine/Diazepam (Valium)	Mice: 100/5 Rats: 40/5	IP	minor cardiorespiratory depression	7278122
	Ketamine/Xylazine (Rompun)	Mice: 80-150/7.5-16 Rats: 40-80/5-10	IP, IM	cardiorespiratory depression (MAP & CO), arrhythmia	15155266, 7278122
	Ketamine/Midazolam	Mice: 50-75/1-10 Rats: 60/0.4	IP	decreased MAP & CO	16174120
	Ketamine/Acepromazine	Mice: 100/5	IP	minor CV depression, hypotension	23382271
	Ketamine/Xylazine/Acepromazine	Mice: 100/2.5/2.5 Rats: 40/8/4	IP, IM	good MAP & HR	11924805
	Pentobarbital Na (Nembutal[2])	Mice: 30-90 Rats: 30-60	IV, IP	decreased CO, MAP & HR; increased ESV & EDV	15155266, 15027618
	Tiletamine/Zolazepam (Telazol)	Rats: 20 - 40	IM, IP	good CI, minor cardiorespiratory depression	17343357
	Thiamylal (SuritalR)	Rate: 25 - 50	IV, IP	cardiorespiratory depression, arrhythmia	1637605
	Thiopental Na (PentothalR)	Mice: 30-40	IV, IP	Cardiorespiratory depression, decreased BP	18172330
	Etomidate	Mice: 22-25	IP	decreased HR, good CO & MAP	7278119, 12814659
	Urethane[1]	Mice: 800 - 1300	IP	good MAP, & CO	15155266
	Urethane/Etomidate/Morphine[1]	Mice: 750/20-25/1-2	IP	good MAP & CO	15604134
	Tribromoethanol[1] (TBE or Avertin)	Mice: 250 Rats: 150	IP	moderate cardiopulmonary depression	16884172
Anesthetics - Inhalant[3]	Isoflurane (Forane)	Mice: 0.1-1.5% Rats: 0.25-2.5% in pure O_2 maintenance	Inhalation	Vasodilation, decreased BP, good CO	18550865, 12003817, 22492676
	Desflurane	To effect (4-6%)	Inhalation	Vasodilation, decreased BP	22929732
	Sevoflurane	Rats: 3.5-4% in pure O_2 maintenance	Inhalation	Vasodilation, decreased BP	21778336, 22167771

*SC=subcutaneous, IM= intramuscular, IP= intraperitoneal, PO=orally, IV=intravenous, HR = heart rate, SV = stroke volume, MAP= mean arterial pressure, CO= cardiac output, CI= cardiac index, ESV = end systolic volume, EDV = end diastolic volume,

1. Terminal Studies only.
2. Dilute stock solution to accurately dose animals
3. These agents should be used only in ways that prevent exposure to personnel. Induce anesthesia in a closed container and maintain with a nose cone in an appropriately ventilated hood.

Rodent Anesthesia Guidelines Cont.

ANESTHESIA TIPS & CONSIDERATIONS

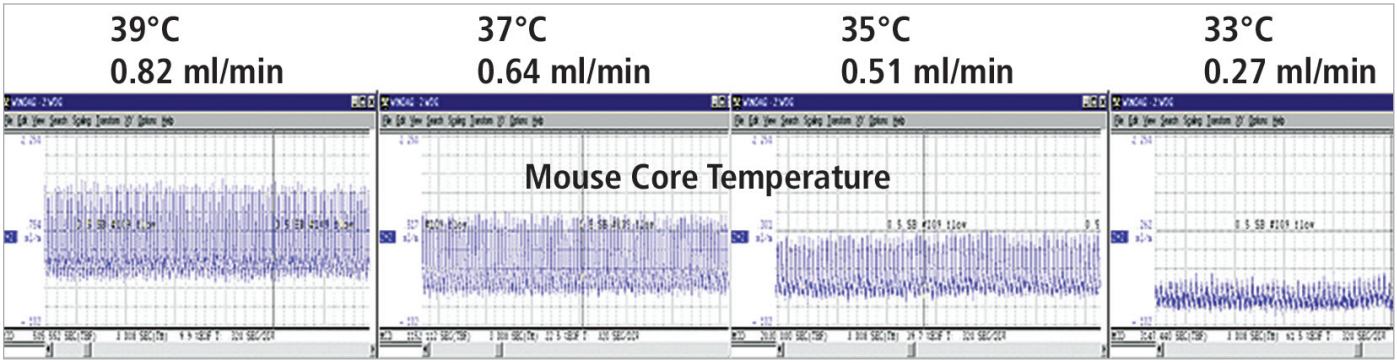
- When anesthetizing post-MI animals, maintenance of coronary artery pressure helps limit tachycardia.
- Induction of anesthesia can cause arrhythmias (junctional rhythms). Treat by reducing the dose of inhalation anesthetic or administering an anticholinergic.
- Halogenated volatile inhalation anesthetics (isoflurane) should be used in a vented hood to reduce operator exposure during procedures.
- It is advisable to monitor blood gases before, during and after anesthesia to ensure normal metabolism and prevent the development of alkalosis or acidosis.
- Anesthetized animals do not completely close their eyelids. Therefore, they are at risk of corneal desiccation and ulceration. It is advisable to protect their eyes with sterile eye-lubricating ointment, especially in long-duration studies.
- It is recommended to use a single injection while delivering an injectable anesthesia to small rodents to reduce anxiety and ensure a stress-free induction and recovery. However, care must be taken when mixing agents for a single injection to ensure safety and efficacy.

CATEGORY	AGENT	DOSE (MG/KG)	FREQUENCY	ROUTE
Anticholinergics	Atropine	Both: 0.02-0.05	Both: once at induction	SC
	Glycopyrrolate	Both: 0.01-0.02	Both: once at induction	SC
Analgesic (NSAID)	Acetaminophen	Rats:100-300 Mice: 300	Rats: 4 hrs Mice: daily	PO
	Aspirin	Both: 100	Both: 4 hrs	PO
	Carprofen	Both: 5	Rats:12 hrs Mice: daily	SC
	Flunixin	Both: 1.1-2.5	Both: 12 hrs	SC, IM
	Ibuprofen	Both: 7.5	Both: daily	PO
Analgesic (Opiate)	Butorphanol	Rats: 0.05-2 Mice: 0.05-5.4	Both: 2-4 hrs	SC
	Meperidine	Both: 10-20	Both: 2-3 hrs	SC, IM
	Morphine	Both: 10	Both: 2-4 hrs	SC, IM
	Pentazocine	Both: 10	Both: 3-4 hrs	SC

Body Temperature

The high metabolic rate and high surface-to-volume ratio of mice means that they lose heat very quickly. It is therefore imperative to avoid anesthetics such as barbiturates, which alter the animal's ability to maintain core temperature (see PMIDs 18172330,15155266, & 15027618).

Similarly, the animal should be warmed during operative procedures which open a body cavity and expose even greater surface area to ambient temperatures for heat loss. Body temperature should be monitored during heating to avoid increasing body temperature above 38°C.



Effect of Core Temperature on Femoral Blood Flow in a 22 gram CD-1 Mouse: As the effect of progressive lower core temperatures in the respective flow traces demonstrates, temperature has a profound effect on femoral blood flow and must be monitored.

Data, courtesy of M.F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

transonic
THE MEASURE OF BETTER RESULTS.

Rodent Anesthesia Guidelines Cont.

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IDEAL ANESTHETIC AGENT

- Reliable
- Wide safety margin
- Rapid onset/rapid recovery
- Easy to administer & control
- Nontoxic
- Causes no physical impairment
- Produces analgesia and muscle relaxation

ACKNOWLEDGEMENT

Thomas L. Smith, Ph.D., Bowman Gray School of Medicine, Dept. of Orthopedic Surgery, Wake Forest University, Winston-Salem, NC.

Application-based Suturing and Suture Selection

INTRODUCTION

Closing a wound, inserting and securing a pressure catheter in a vessel, relieving tension on the catheter cable or just fixing the aorta on the aortic cannula during ex-vivo heart experiments all require the usage of suture material. To select the appropriate suture for a given technique requires knowledge of suture products, suturing experience and relentless practice.

Surgical techniques as well as suture materials are continuously improving. Each suture material has its own absorption profile. Tensile strength and surface coating are constantly improving leading to better resistance to infection with less tissue injury (nonallergenic and noncarcinogenic profile).

APPLICATION-BASED CHARACTERISTICS

Every application requires specific features of the suture material. The six characteristics to check for each application are 1) uniform tensile strength, 2) diameter, 3) ease of handling, 4) performance of a surgical knot and 5) ability to be sterilized without losing its mechanical characteristics. Lastly 6) the material should have no irritating components that would provoke excessive tissue reaction.

Other characteristics to consider per application are:

Available sizes, absorbability (natural vs synthetic), structure (mono vs. multifilament), elasticity, fluid absorption, capillarity, breaking strength, knot-pull tensile strength, knot strength, memory, plasticity, pliability, ease of handling of suture material, biocompatibility.

In this workbook we will cover 3 applications where suture techniques are required.

1. Inserting and securing a pressure catheter in a rodent blood vessel
2. Suturing to relieve tension on the cable of an implanted flow probe
3. Suturing of the aorta on the aortic cannula (mounting) in preparation of isolated heart experiments

INSERTING AND SECURING A PRESSURE CATHETER IN A RODENT BLOOD VESSEL

Suture material size should be based on target vessel diameter. In a rat surgery, USP 4-0 or smaller should be used when performing catheter insertions in for instance the carotid or femoral artery. When the aorta is cannulated a larger size can be used.

In mouse applications, catheter insertion in the peripheral zones require USP 6-0 or smaller. The application note femoral artery mouse pressure measurements described in this book uses 7-0. The more experience one gains, the easier it is to work with thinner sutures. For mouse aortic work 5-0 or smaller should be used.

During ligation of a blood vessel, both mono and multifilament (braided, twisted material) can be utilized. Multifilament material is however preferred as it has a greater tensile strength, better pliability (less stiff) and flexibility while directly ligating the vessel. When tying a knot with a monofilament suture, one strand can easily crush or crimp the target vessel while the knot can come undone, increasing chances of bleeding. Braided multifilament suture material is preferred because it can be easily tied off, however one should pay attention to suture tissue-friction when passing suture under the small vessels. The friction between the suture and vessel wall can create bleeding or undesirable twisting of the vessel. To prevent this, some multifilament sutures are treated with special coatings to simplify its passage and to reduce tissue-friction induced damage. When pulling suture under the vessel, vascular access should be cleaned from adventitia as the vessel lumen can twist making catheter insertion challenging and sometimes even impossible with the risk of breaking the catheter on insertion.

Application-based Suturing and Suture Selection Cont.

SUTURING TO RELIEVE TENSION ON FLOW PROBE CABLE

For this type of suturing, the chosen material and thickness should match the flow probe cable thickness. As the flow probe cable is durable both mono and multifilament suturing material can be used, however multifilament is preferred. With monofilament material, one should pay attention to tensile strength as over-tied monofilament sutures can cut into cable, creating weak points. This risk does not exist when using braided multifilament sutures. In addition, there is no risk of fluid imbibition or bacterial growth as it is an acute setting. Also, multifilament material has much better pliability (less stiff) and flexibility when directly placed on the cable. Suture tissue-friction on the flowprobe cable when relieving cable tension on the target vessel is not problematic in this application.

SUTURING OF THE AORTA ON THE AORTIC CANNULA (MOUNTING) DURING EX-VIVO PREPARATION

Lastly, to secure a rodent heart aorta onto the perfusion cannula, it is important to chose a material that is good to handle when wet. The suture knot should be directly positioned into the groove of the cannula and not weaken when the perfusion fluid is flowing through the system. Braided multifilament material is a good choice in this situation. Suture size for a mouse heart set-up would be 5-0 or smaller, while 6-0 or smaller is suitable for a rat heart set-up.

Note: some application notes discussed in this workbook discuss other suturing material in e.g. securing endotracheal / tracheal tube in exposed mouse trachea after tracheotomy. Multifilament, braided material is preferable over monofilament as multifilament is not likely to easily crush the trachea when over-tied. A 3-0 and thinner is recommended for this application. Please pay attention that braided suture material does not twist the trachea and that the tracheal tube can be easily inserted during this maneuver.

SUTURE RECOMMENDATIONS

APPLICATION	MONOFILAMENT	BRAIDED MULTIFILAMENT	RAT SIZE	MOUSE SIZE	OTHER COMMENTS
Vessel Catheterization	Can be used but knots may be insecure and lead to bleeding; tying may crush small vessels.	Preferred: greater tensile strength & flexibility. Caution against friction abrasion & twist of vessel with passage.	≤ USP 4.0	≤ USP 6.0	Some Braided Multifilament Sutures are coated for easy passage.
Aortic Catheterization			≥ USP 4.0	≤ USP 5.0	
Flowprobe Cable Strain Relief	Overtied suture can cut into cable causing weak point in probe wire.	Preferred: no risk of fluid imbibition or bacterial growth for acute prep	Choose apropriate to probe cable size		
Ex Vivo Aortic Cannula	Not recommended.	Preferred: Easy to tie and maintain knot in cannula groove.	≤ USP 6.0	≤ USP 5.0	Use suture easy to handle when wet

Why Measure Flow? The Flow and Pressure Relationship

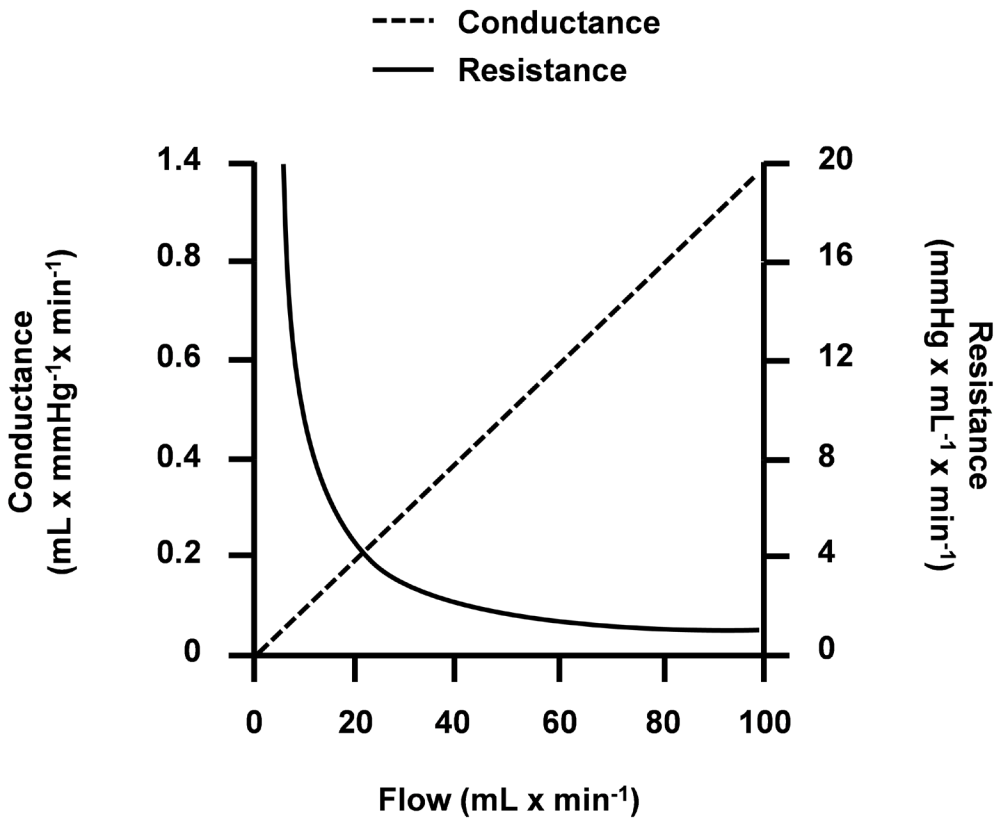
Adequate blood flow provides nutrients and oxygen to tissues within a specific optimum ratio between oxygen delivery and oxygen consumption. Individual tissue requirements vary between tissue types and conditions (physiological and pathophysiological) while concurrently are modulated and affected by systemic and local mechanisms. Although arterial blood pressure provides a sufficient pressure gradient to drive blood flow throughout the arterial system at any one time, the local regulation (via local arteriolar tonus) can participate to ensure adequate flow to meet highly specific local demands with some degree of systemic independence.

RESISTANCE AND CONDUCTANCE

A more comprehensive hemodynamic assessment should consider both pressure and blood flow and foremost the interaction between them. The pressure and flow relationships used to quantify changes in vasomotor gradient are resistance (pressure gradient/ flow) and conductance (flow/pressure gradient).

$$\text{Resistance} = \frac{\text{Pressure}}{\text{Flow}} \qquad R = \frac{P}{F} \qquad \text{Conductance} = \frac{\text{Flow}}{\text{Pressure}} \qquad C = \frac{F}{P}$$

Based on the formulas, these variables are opposite yet not interchangeable.



Vascular tone plotted as resistance or conductance at constant driving blood pressure of 100 mm Hg with flow varied over physiological ranges (1).

The Flow and Pressure Relationship Cont.

When perfusion pressure is held constant, conductance (flow/pressure gradient) is linearly related to blood flow; while resistance (pressure gradient/flow) is inversely and, therefore nonlinearly, related to blood flow. (1). The linear nature of the conductance response enables simple mathematical means to be calculated for variable responses. The nonlinear relationship between the primary changing variable, flow, and calculated vascular resistance is dramatically emphasized at low flows, induced, for example, by vasoconstriction (1).

Data misinterpretation can range from minor inaccuracies in parameters with small variability, to gross errors that may completely conceal the real biological responses. A demonstration of opposite conclusions can be found, and better understood, in a study evaluating thermal status and baroreflex regulation of regional circulation (2). Please consult the supporting literature for more details.

Traditionally, vascular tone and changes in vascular tone are expressed as vascular resistance and percentage change in resistance. So, based on the points mentioned above, **which index of the vasomotor response is the most appropriate choice to investigate the flow and pressure relationship? Resistance or Conductance?**

One approach is to consider the parameter that undergoes the primary change (flow or pressure). The proper index, resistance or conductance, to accurately reflect changes in vascular tone, should have the parameter (flow or pressure) that undergoes the primary or significant change as the numerator (1). This is a relatively straight forward method when applied to isolated organ preparations with pressure or flow held constant. However, when conducting in vivo studies, specific tissue perfusion exhibits dynamic flow and pressure behaviors. Therefore, the appropriate parameter, conductance or resistance, needs to be selected carefully (3). For example, conductance seems to be the more accurate measurement when analyzing baroreflex responses, even with pressure changes surpassing flow changes (2).

From the physiological point of view, it is helpful to understand that local changes in vascular tone primarily produce changes in local blood flow rather than systemic blood pressure. Under most conditions in vivo, local vascular responses will result primarily in flow responses rather than pressure responses and under those conditions conductance would be the more suitable choice as vascular tone index (1).

Using the analogy between hemodynamics as an hydraulic system when analyzing regional vascular responses; if total system flow (Cardiac Output), system pressure (SAP) and local flow (specific organ/tissue flow) are known, then the change in pressure associated with the regional vasomotor response can be calculated using either resistance or conductance indexes. However, since these measurements may be technically impossible in many preparations, especially in vivo, the selection of the appropriate index to evaluate the underlying physiological mechanism becomes even more important (3).

Cardiovascular responses are usually multi-layer systems, with inter-dependencies and opposite and redundant subsystems aiming to maintain homeostasis under physiological and pathophysiological states. Cardiac, vascular, and central effects must always be considered in experimental design and data interpretation. The cardiovascular variable in focus should always be considered against other variables, especially when cardiovascular maneuvers (using pharmacological agents for example) are performed in vivo. Vasomotor, pressor and reflex responses should always be well-thought-out. These effects could be present, not only under baseline conditions but also once the system is challenged, albeit in potentially different degrees.

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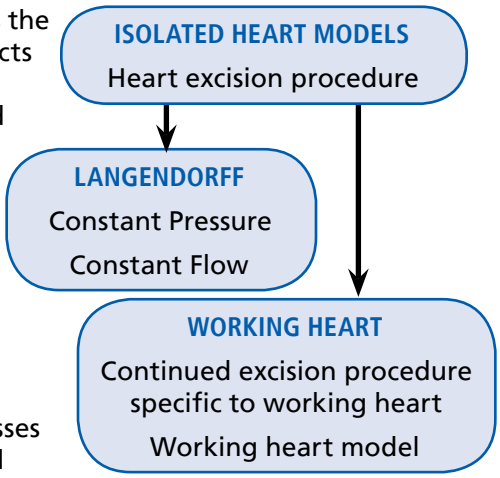
Isolated Heart Models for Cardiac Assessment



The practise of isolating the heart from an animal's circulation simplifies the examination of inotropic (contractile) and chronotropic (heart rate) effects without confounding vascular responses. As isolated heart models lack fresh blood circulation, hormonal and autonomic nervous responses and otherwise very complex in-vivo factors are decoupled which helps to perform a variety of basic analyses of fundamental cardiac properties. This allows for testing of pharmacological compounds, unmasking potential direct action of studied compounds or studying basic cardiac muscle physiology. The preparation also allows direct inductions of ischemia or arrhythmias with precise mapping of the conduction pathways, coronary blood flow regulation and cardiac metabolism.

There are two basic types of isolated heart models:

- Langendorff: retrograde perfusion via the cannulated aorta, flow crosses the aortic valve to fill the Valsalva sinuses then enters into the left and right coronary arteries through the left and right coronary ostia. Perfusion buffer then passes through the coronary vascular bed before draining into the coronary veins and coronary sinus in the RA. In this preparation the ventricular chamber(s) are not perfused. This set up is very important to discern mechanical behavior of smooth vascular muscle cells in the coronary vasculature, expressed as changes in vessel radius. Set-up can either use constant pressure via gravity-fed apparatus or constant perfusion flow rate (1).
- Working Heart: antegrade perfusion where perfusate enters through the mitral valve and is ejected through the aortic valve. This major modification in the isolated heart model was made by Neely and Morgan in 1967 (12). Isolated heart preparations that perform mechanical work are commonly referred to as the "working heart," but a more appropriate term is the "ejecting heart" as the Langendorff heart is also "working" (13). See "Working Heart Model for Cardiac Assessment" for more information.

Before we discuss the two types of isolated heart models, we will go over the procedure of how to exceed the heart correctly in preparation of an isolated heart protocol.

EXCISION OF THE HEART AND ITS CANNULATION (MOUSE)

Mice are given a intraperitoneal injection of heparin (40U) 15 min prior to harvesting the heart. Excision is done under general anesthesia using median sternotomy and the heart is harvested together with the lungs and immediately placed in ice-cold perfusate (4°C) to arrest beating. Subsequently, for Langendorff set-up the lungs and excessive tissue are removed; for working heart leave on to remove later (as discussed on page 52). The ascending aorta is then fixed on the aortic cannula. Gauge sizes of cannulas for mouse range from 22-16, for rats from 14-8 (1) and the cannula is opened to start a full retrograde buffer flow. The heart should start beating vigorously. If not, the cannula may be occluding one or both of the coronary arteries. If this is suspected, reposition the cannula away from the coronary arteries. If the heart distends and does not beat, the cannula may be across the aortic valve. If the coronary artery leaks (perfusate sprays from the aortic

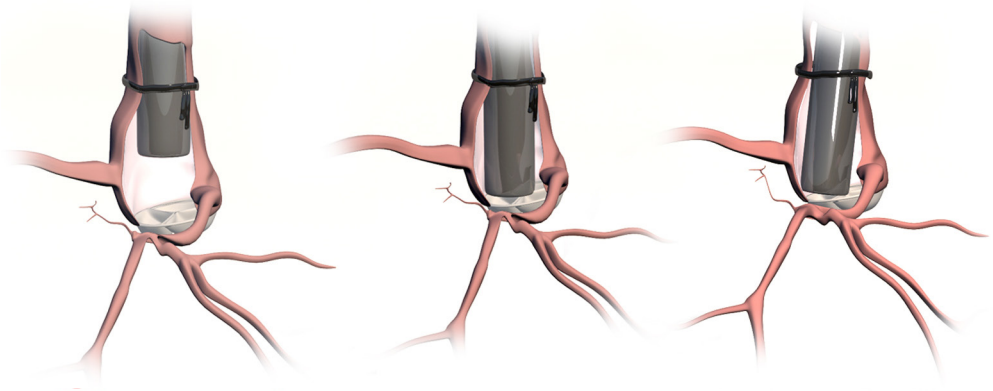
SUGGESTED GUIDELINES BY SPECIES

SPECIES	HR (BPM)	LVV*	CORONARY FLOW RANGE	FLOWSENSOR SIZE	PRESSURE CATHETER SIZE
Mouse	450-550	20 µl	2 - 8 ml/min (16)	1PXN or 2PXN	1.2F
Rat	330-360	0.1-0.2 ml	7 - 14 ml/min (15)	2PXN or 3PXN	1.6F (1.9F)

*LVV values are for fluid filled balloon inserted in to LV

Langendorff Heart Model for Cardiac Assessment Cont.

root), advance the cannula closer to the aortic valve (this phenomenon can occur if a brachiocephalic artery is cannulated in place of the ascending aorta). The perfusate is typically a nutrient rich oxygenated buffer such as a Krebs- Henseleit or Tyrode's buffer, pH 7.4, conductivity of 16-20 ms/cm. Right ventricle thermocouple wire can be inserted to monitor cardiac temperature (11). The first minutes are critical in determining the success of the preparation. Experiments can start 10-15 min after successfully establishing heart beats and can last up to 4 hours. In most cases investigation will be time-limited as non-blood perfusion invites protein loss leading to tissue edema. Pacing can be used to increase heart rate to physiological levels allowing better direct comparisons with in-vivo cardiac contractility. For Langendorff set up, there are two options 1) constant flow up to 15 ml/min/g or a constant hydrostatic pressure (60-80 mmHg with commencement between 50-60 mmHg). These two options are discussed in detail after the upcoming section.



Aortic cannula with groove correctly placed in the aortic arch and ligated

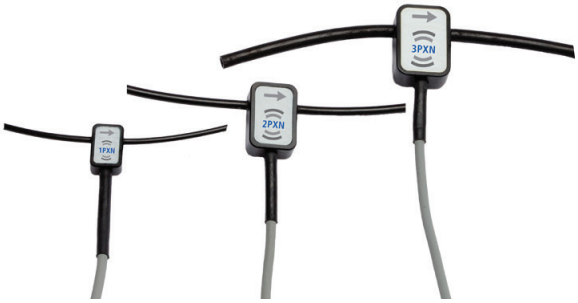
Aortic cannula with groove incorrectly placed in the aortic arch

Aortic cannula with groove incorrectly placed in the aortic arch

Correct aortic cannula placement (on the left) when heart is mounted as compared to incorrect cannula placements (on the right). The difference is in the ability to perfuse both coronary sinuses with similar hydrostatic pressure to ensure the viability of perfused tissue.

Langendorff Model

The Langendorff preparation is widely used for species-specific pathways using hearts isolated from all mammalian species. Selection of the model heart is very important, especially when a variety of genetically modified strains are available (4-7). The most common prepped hearts are from mice, rats, guinea pigs, ferrets and hamsters.



Transonic PXN Inline Flowsensors insert into the tubing circuit to allow for direct measurement of volume flow into the heart. This ensures that the heart is receiving an adequate amount of nutrients.

INTRODUCTION OF FLUID-FILLED BALLOON INTO THE LV TO TEST MAX DEVELOPED PRESSURE AND PRELOAD

A fluid filled balloon is introduced into the LV through a cut in the atrial appendage, then the balloon is passed through the mitral valve and inflated to yield a constant end-diastolic pressure (EDP). The balloon has to be very compliant with high frequency response (11). The balloon is filled with saline using a 3 way stopcock and syringe. It is important to monitor the balloon and calibrate periodically as it can deteriorate over time. A new balloon should be introduced if it is not performing adequately.

When the balloon is in position, it should be inflated to pre-stretch myocardial fibers using up to 10 mmHg pressure. The preload on the balloon should be increased gradually while monitoring ventricular developed pressure (LVdp). Every increment of balloon pressure should be followed by an assessment of maximum developed pressure and systolic pressure. By adjusting the balloon volume, the left ventricular diastolic pressure is set at

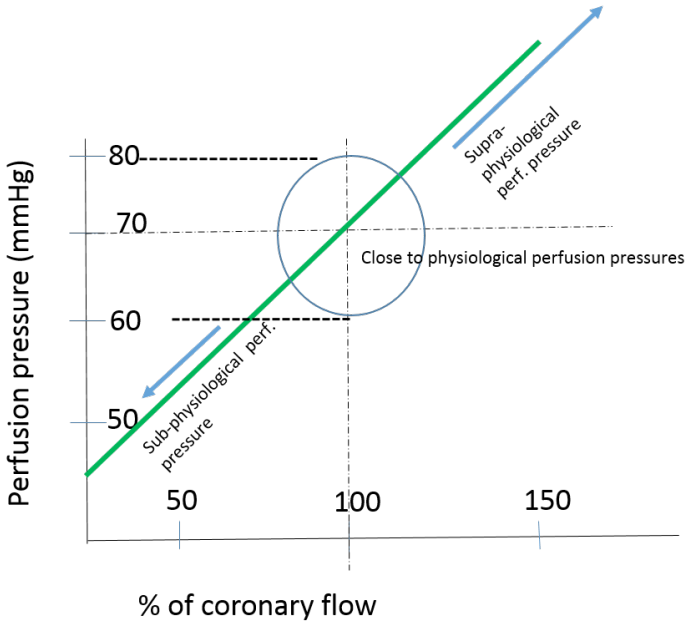
Langendorff Heart Model for Cardiac Assessment Cont.

5-10 mmHg in mice (14), 8-10mmHg in rats, while a physiological normal systolic pressure should be maintained.

A constant pressure is set using gravity and elevation of the aortic bubble trap compliance chamber (1 mmHg = 13.6 mm of water column). As LVdp causes the aortic valve to shut, it forces the perfusate into the coronary arteries. In this mode, the aortic valve prevents the solution from entering the LV, but coronary arteries are still perfused through coronary ostia located outside of the valve, maintaining the viability of myocardium.

Care should be taken to not penetrate the aortic valve and position the aortic cannula such that it can perfuse both coronary ostia. Perfusate drains as effluent from the coronary circulation via coronary sinus and pulmonary artery and tends to drip from the apex where it is collected. This system set up provides basic application for physiological monitoring and/ or cardiomyocyte isolation.

- The mounted heart should be perfused by flow of up to 15 ml/min/g of heart tissue.
- Initial perfusion pressure should be about 50-60 mmHg, and can be increased to 100 mmHg.



Example: relationship of perfusion pressures and coronary flow in Langendorff-perfused heart. Coronary flow is expressed as percent of coronary flow at the normal physiological perfusion pressure (typically 60-80 mmHg).

A pilot study should be run to test physiological parameters prior to the actual experiment. The pilot experiment establishes the baseline coronary flow in relation to the driving perfusion pressure through the system at a given flow rate. The relationship is animal, species, and strain-specific. Usually this relationship is linear with the correlation coefficient in the higher nineties (14).

CONSTANT PRESSURE LANGENDORFF

Pressure is held constant (fixed known pressure) and changes in coronary resistance are detected as changes in blood flow (changes measured volumetrically). Constant pressure is achieved by elevating the aortic bubble trap compliance chamber. Typically ~1 m of elevation above the isolated heart is recommended as 1 mmHg = 13.6 mm of water column therefore 0.816 m would be needed to create 60 mmHg. By using a syringe pump or multiple reservoirs, different compounds in a variety of concentrations can be delivered to or collected from the heart for pharmacological dose response studies. A flow sensor can be added after the oxygenator/heater/ bubble trap and a solid state pressure catheter can be introduced to measure perfusion pressure. See drawings for more details.

CONSTANT FLOW LANGENDORFF

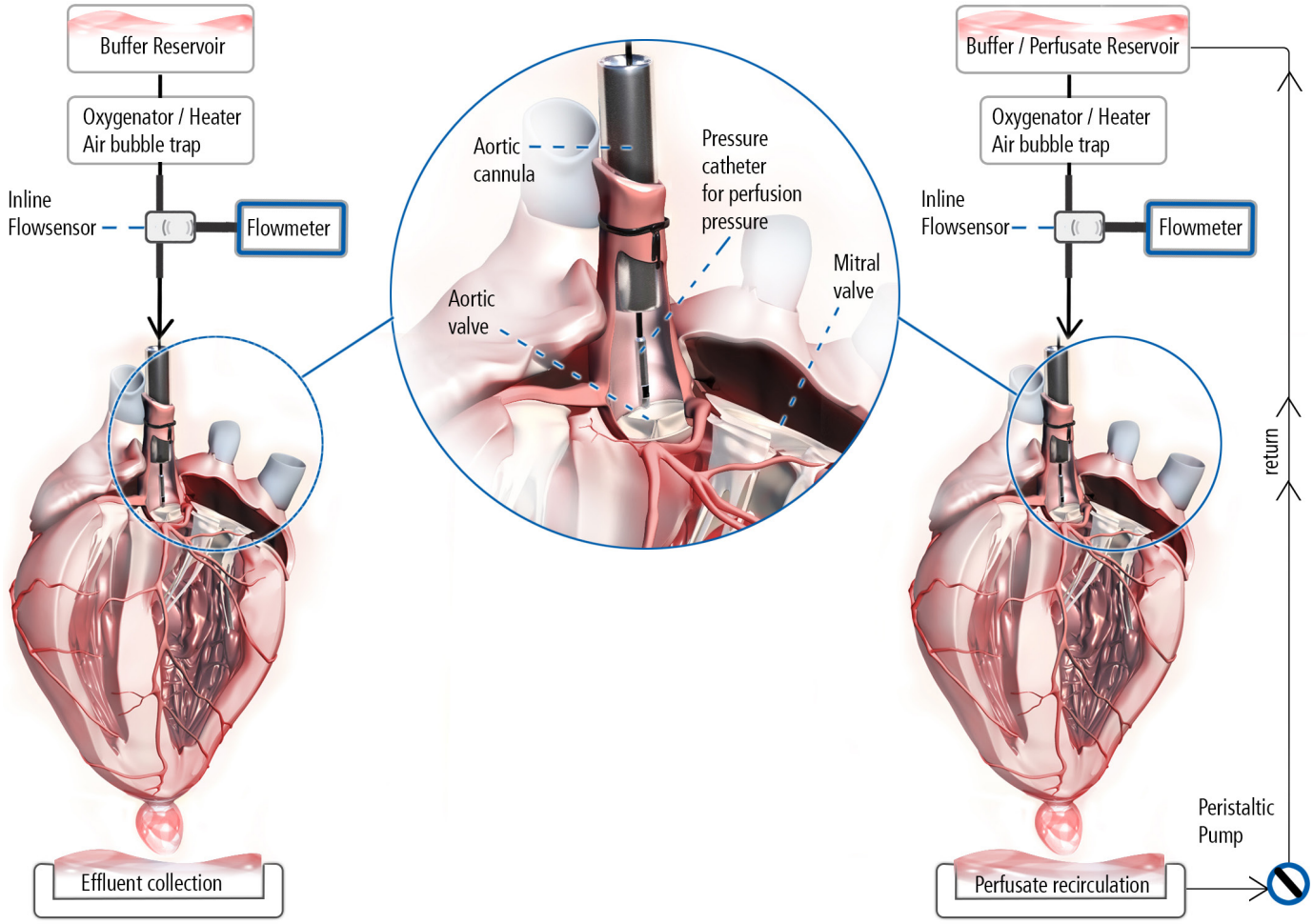
Flow is held constant at fixed (known) flow rate via a peristaltic pump and changes in coronary resistance are detected as changes in pressure. This set up is predominantly used when studying coronary vascular tone (vasoconstrictors, vasodilators), and smooth muscle or endothelial function. The peristaltic pump is usually set to deliver perfusate at 1.5 to 2 times of anticipated flow rate. The following formula is based on heart weight and is used to calculate coronary flow in constant flow Langendorff (8).

Coronary flow = $7.43 * HW^{0.56}$

Langendorff Heart Model for Cardiac Assessment Cont.

Setting the flow rate high allows the return of buffer to supply the reservoir. Fluid delivery rate is a function of the tubing size and pump rotations per minute. Bubble trap elevation should not be more 100 mm from the heart. A pressure transducer can be used at the aortic cannula. If using the fluid filled balloon, the 3 way stopcock with valve could also accommodate a pressure catheter.

Please note: When using inotropes or during arrhythmias, release of adenosine by the coronary arteries creates a condition of high oxygen consumption while decreasing coronary resistance with a reduction in coronary flow. In the constant flow set up, the heart may not be able to autoregulate coronary flow as compared with the constant pressure system. The resulting increases in cardiac work may cause severe ischemia and more cardiac insult than the constant pressure set up. Additionally, pressure read outs should be within species specific ranges.



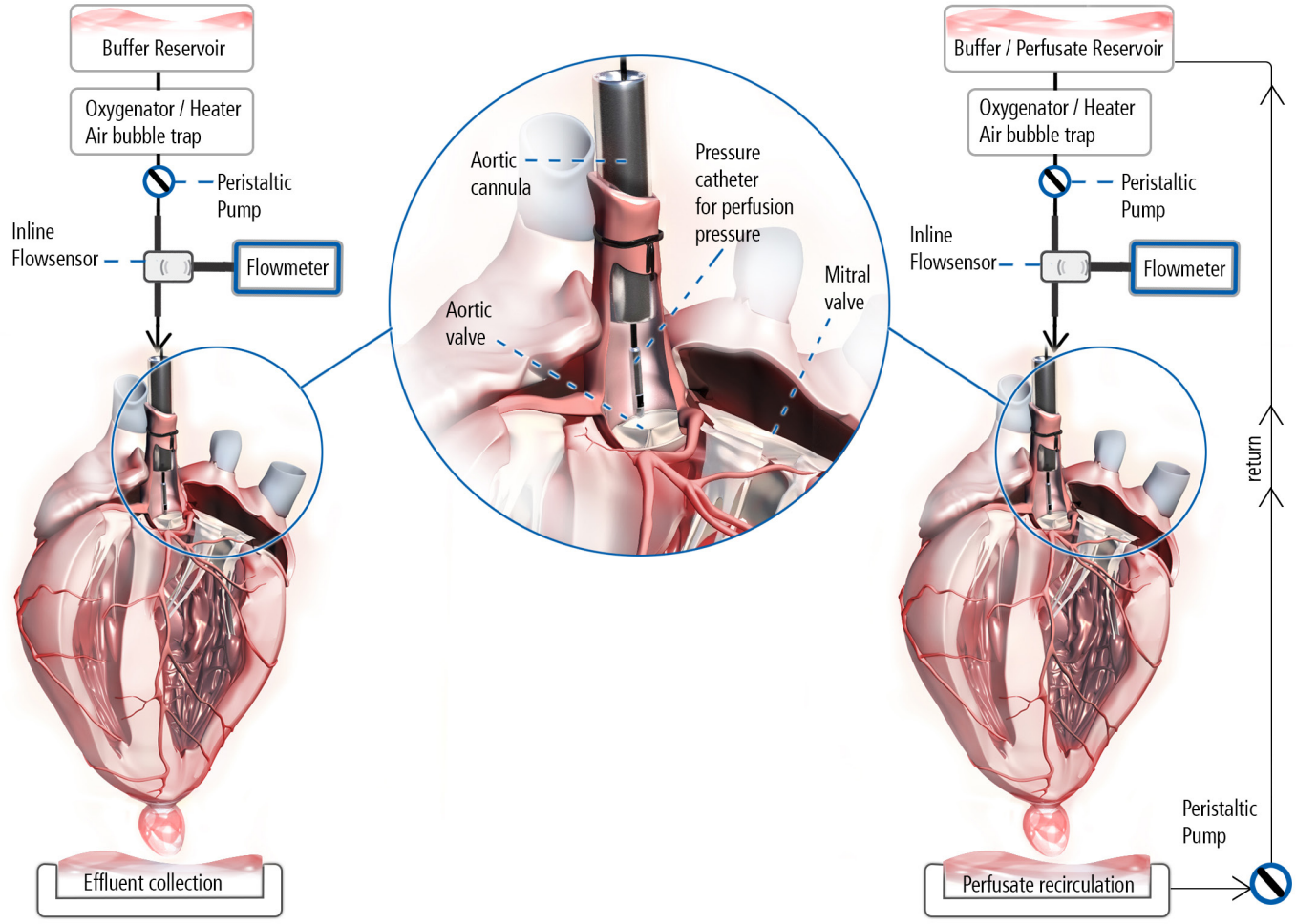
Drawing on the left shows buffer reservoir and collection of coronary flow as effluate drains from coronary sinus and pulmonary artery. On the right the effluate is recirculated using a peristaltic pump (e.g. drug compound testing application). The inline volume flow sensor helps to determine the LV preload (ml/min). The solid state pressure sensor can be used to measure inflow (perfusion pressure). If using the fluid filled balloon, the 3 way stopcock with valve could also accommodate a pressure catheter (not shown) to measure and record the LVdP (Left Ventricular developed Pressure).

Langendorff Heart Model for Cardiac Assessment Cont.

CONSIDERATIONS CHOOSING BETWEEN CONSTANT PRESSURE AND CONSTANT FLOW PERFUSION

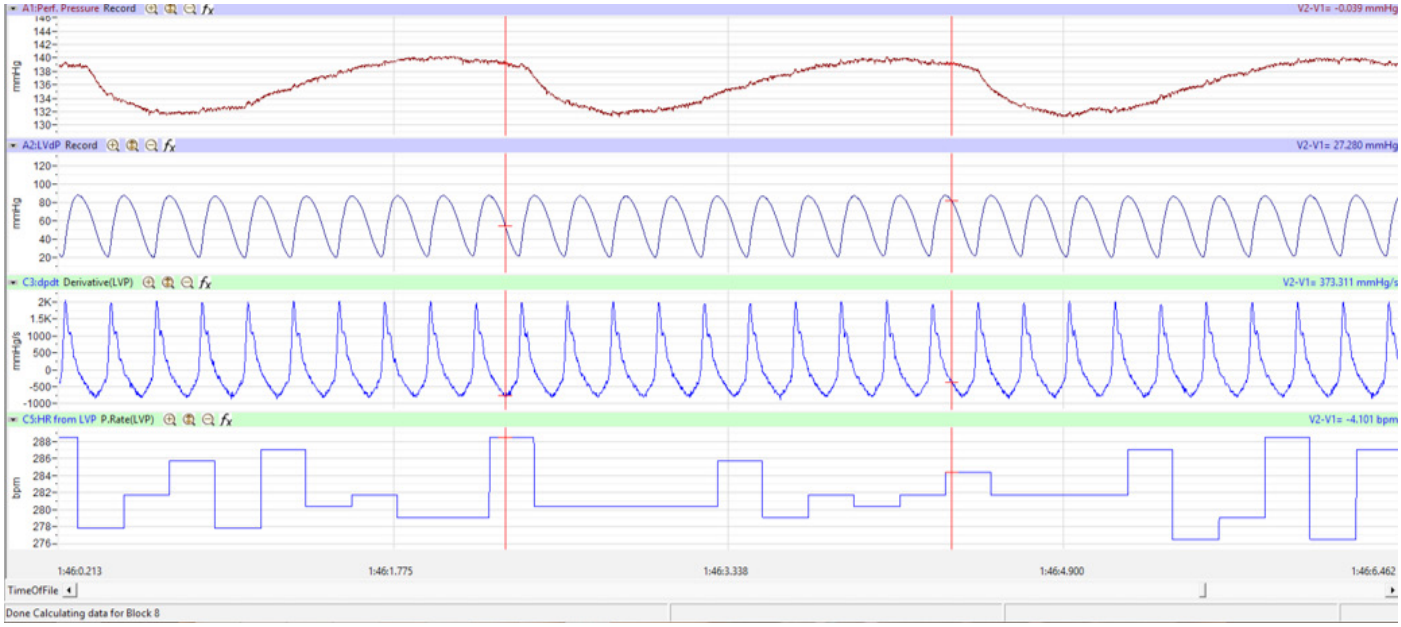
Both perfusion systems, when using non-blood perfusate, have coronary flow rates higher than in-vivo (8-12 ml/min/g of heart) (3). The constant flow system may override the auto-regulatory cardiac mechanisms otherwise guarded by pressure development in the system. Additionally, constant low flow rates supplied by the roller pump into coronaries may not develop into sufficient pressure, resulting in overall low-cardiac perfusion.

Limitations of the Langendorff prep include induction of arrhythmias (especially in larger hearts), cardiac arrests, regional ischemia (anoxia, hypoxia) based on limited coronary flows, and a 5-10% per hour deterioration of chronotropic and contractile function (3). To limit the coronary blood flow insufficiency, switching between a constant pressure and constant flow mode is desirable when working with the Langendorff apparatus.

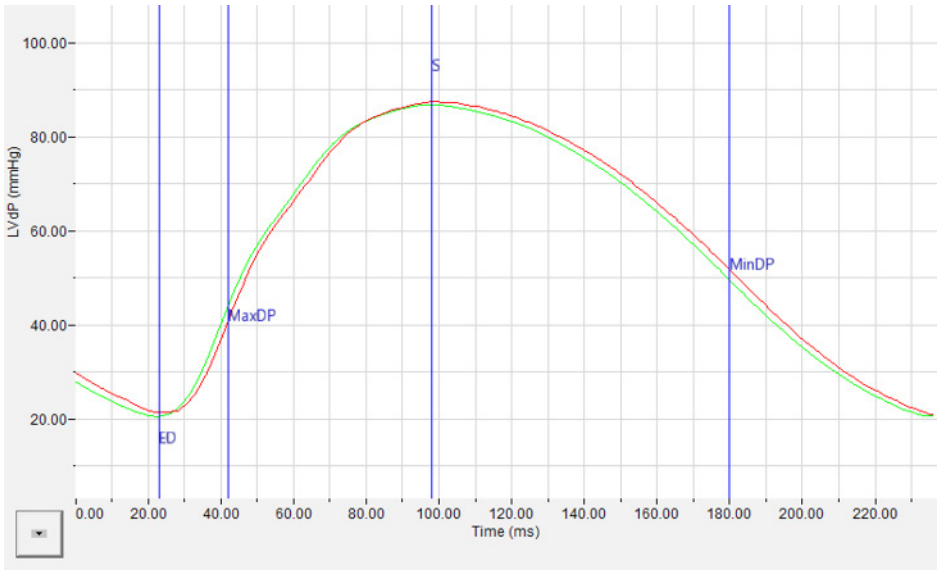


Basic set up of constant flow Langendorff. On the left, a peristaltic pump after the oxygenator/heater/ (bubble trap) provides constant flow. Inline flow sensor can be added to enable measuring the flow rate of buffer or perfusate/effluate. Depicted on the right, effluate is collected as it drains from the coronary circulation via coronary sinus and pulmonary artery and drips from the cardiac apex and is recirculated back into the reservoir using second (return) peristaltic pump. Please note the location of pressure catheters. Pressure measurements are important in this set-up to help adjust the flow rate/speed of the roller pump.

Langendorff Heart Model for Cardiac Assessment Cont.



(Above) Data from the rat constant flow Langendorff system configuration. Channel 1 records perfusion pressure, while channel 2 captures LVdp. Channel 3 is set up to capture live dPdt, the derivative of LVdp, and the last channel captures HR.

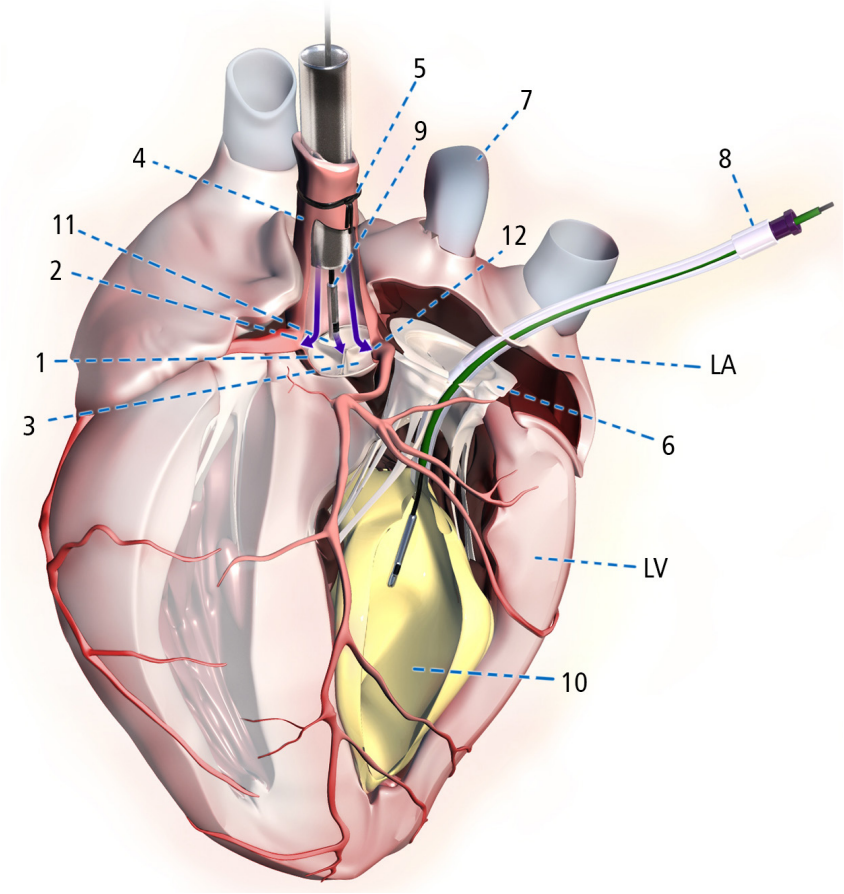


(Right) Data from the rat constant flow Langendorff system configuration LVdp trace with end diastole location (ED); dp/dt max (MaxDP); peak systolic pressure (S); and dp/dt min (MinDP) displayed.

	HR BPM	DURS MSEC	DURD MSEC	DURC MSEC	PMAX MMHG	PMIN MMHG	DPMAX MMHG/ SEC	DPMIN MMHG/ SEC	PMEAN MMHG	PH MMHG	ESP MMHG	EDP MMHG	TAU M MSEC	TT PEAK MSEC
Mean	281.8	149.8	62.8	212.9	87.1	20.7	1868.2	-773.9	58.8	66.4	62.3	20.8	21.5	74.2
SD	1.7	3.5	3.2	1.0	0.4	0.5	10.7	10.0	0.5	0.2	12.2	0.5	1.5	1.7

Sample of calculated values for rat constant flow Langendorff system using data acquisition software with mean and SD values, performed out of 15 cycles. Systolic duration (DURs), diastolic duration (DURd); cycle duration (DURc); peak pressure (Pmax); min pressure (Pmin); pulse height (PH); Tau Mirsky (Tau M); time to peak pressure (TT Peak).

Langendorff Heart Model for Cardiac Assessment Cont.



Detail of 2 single pressure catheter set-up (one used for detection of perfusion pressure, the other for LVdP)

1. Right semilunar cusp
2. Posterior semilunar cusp
3. Left semilunar cusp
4. Ascending Aorta
5. Suture location (in the groove)
6. Mitral valve
7. Left Pulmonary veins
8. Pressure catheter inserted into fluid filled balloon through Tuohy Borst
9. Pressure catheter measuring perfusion pressure
10. Fluid filled balloon inserted through mitral valve into LV
11. Opening of the right coronary artery
12. Opening of the left coronary artery

TIPS FOR SETTING UP THE SYSTEM

- It is critical to not damage the valve during cannulation.
- Correctly tighten the suture around the ascending aorta (position in the groove of the cannula).
- Properly set up pressure catheters to trace perfusion pressure and LVdP.

DISADVANTAGES OF ISOLATED HEART

- Possibility of ischemic preconditioning of the organ during dissection, mounting and other manipulations.
- Lack of colloid osmotic pressures leading to cardiac edema (8).
- Likelihood of high coronary perfusion pressures with pre-mixed crystalloid solutions (as different from blood or plasma) causing coronary endothelial damage during perfusion.
- Chance of baseline inconsistencies if using perfusate with different Ca^{2+} levels such as a modified Krebs-Henseleit buffer (9).
- Possibility of bacterial contamination of the perfusate causing the exogenous peroxynitrite activation (10).

Langendorff Heart Model for Cardiac Assessment Cont.

Most common reasons why cardiac contraction of the prepped heart fails:

- Miscalculations and inaccuracies in the formulation of perfusate
- Addition of toxic agents to perfusate
- Low temperature of final perfusate
- Bacterial contamination of the perfusate or perfusion apparatus (e.g. stopcocks, valves, aerators, bubble traps etc.)
- Surgical errors during excision
- Incidence of developed and uncontrollable arrhythmias

APPLICATIONS OF LANGENDORFF SET-UP USING MEASUREMENTS FROM PRESSURE TIPPED CATHETERS

- Investigation of positive inotropic effect (pharmacology)
- Investigation of negative inotropic effect (pharmacology)
- Coronary vasculature dilations (pharmacology)
- Gradual determination of hypoxic damage
- Calcium antagonism
- Metabolic studies with nuclear magnetic resonance
- Arrhythmogenic, anti-arrhythmic, anti-fibrillatory effects (pharmacology)
- Electrophysiological evaluation of cardiovascular agents

Langendorff Heart Model for Cardiac Assessment Cont.

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Working Heart Model for Cardiac Assessment

PULMONARY VEIN OCCLUSION AND PREPARATION OF THE PULMONARY ARTERY FOR CANNULATION

Excision of the heart is followed by additional preparation steps in case of a working heart set-up. The purpose of these steps is to create a closed left atrial system to ensure that all volume and pressure from the left atrial block is transmitted to the left heart structures. Failure to completely occlude the pulmonary veins could result in preload deficiency and may falsify results or create an unstable working heart preparation. Resect the right lung distal to the clip. Due to difficulty in dissecting the pulmonary artery free, you may occlude the pulmonary veins to distend the pulmonary artery, making it easier to incise without injuring the nearby structures in a beating heart model. Repeat this procedure for the left lung. Once both pulmonary arteries are occluded, the right atrium will visibly distend and the heart may become bradycardic. This is because the right ventricle becomes pressurized. If this does not occur, it is likely that the pulmonary veins are not completely occluded, and that preload will be insufficient for working heart mode. If the heart is not able to maintain cardiac output after left atrial (LA) cannulation and attempted transition to working heart (see below), consider placing additional clips or a tie around the pulmonary vein stumps to occlude any residual leak. Note that some investigators incise the pulmonary artery prior to ligation of the pulmonary veins to avoid pressurization of the right ventricle.

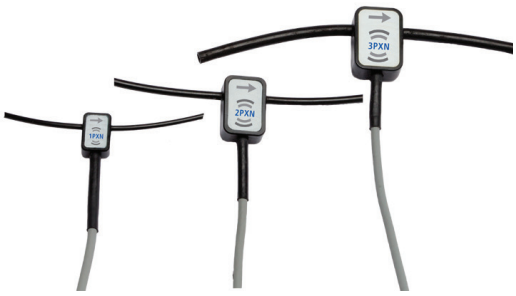
LEFT ATRIAL CANNULATION

Make a small incision in the upper body of the left atrium, approximately 3 mm above the atrioventricular groove. Position the left atrial cannula perpendicular to the plane of the mitral valve and pointed towards the atrial septum. Allow perfusate to flow and ensure that the perfusate is warm to the touch (it gets cold quickly when sitting in any non-jacketed tubing) in order to avoid myocardial dysfunction due to hypothermia following transition to working mode. Transition to a drip rate during cannulation.

Subsequently insert the atrial cannula into the body of the left atrium, taking care not to use excessive force, which can tear the atrium. Assure that the LA cannula is positioned so that it sits in the middle of the atrium without any tension on the atrial wall. The most common reason that the heart becomes mal-perfused upon transition to working heart mode is that the LA cannula abuts the atrial septum, which occludes left atrial inflow.

TIPS FOR SETTING UP THE SYSTEM

1. Ensure that the cannula position does not inhibit flow to the coronaries.
2. Correctly tighten the suture around the ascending aorta (best in the groove of the cannula).
3. Use the pressure sensor and flowsensor to control the preload.
4. Properly set up Pressure/PV Catheter in the LV using the CO from the flowsensor.
5. Temporary preload reduction is performed by clamping of the preload inflow line.



PXN in-line (above) and PXL clamp-on (not shown) tubing flowsensors can be implemented into the circuit giving information about preload or afterload of the isolated ejecting heart.

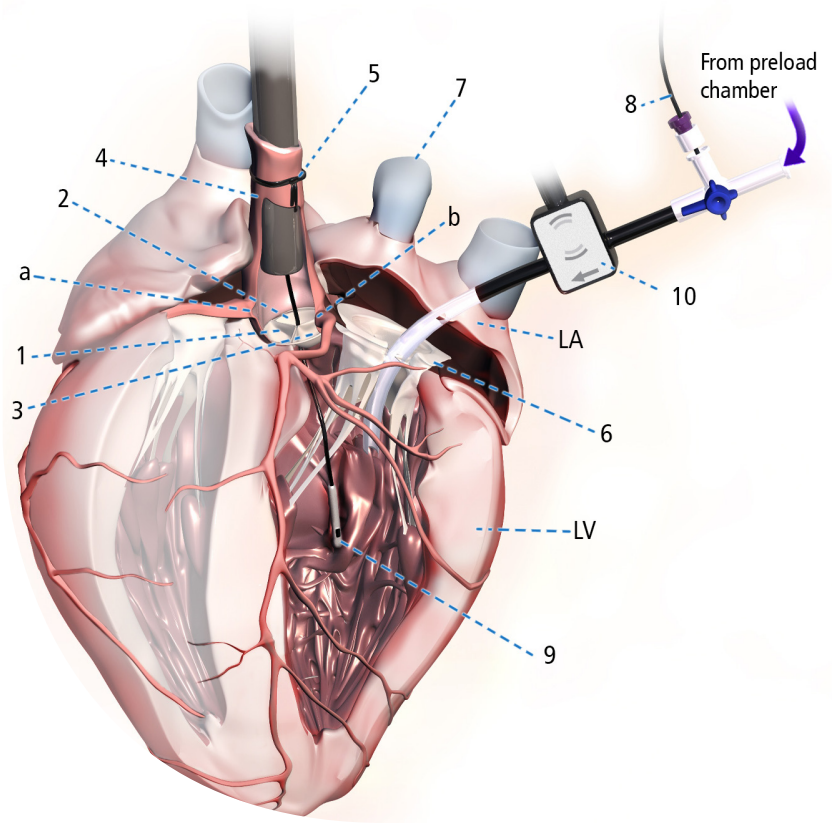
Working Heart Model for Cardiac Assessment Cont.

Working Heart Model

The difference of working heart, also known as isolated ejecting heart (IEH) or fluid ejecting heart model, as compared to Langendorff is in the set-up and parameters measured. In the Langendorff set-up, the only perfused structures are the coronary arteries. In the ejecting heart model the ventricle is also perfused allowing control over both the preload and afterload with the ability to capture complete PV loops. Moreover, load independent parameters are able to be investigated including comparison of pressure-volume area (PVA) to myocardial oxygen consumption (mVO2), using temporary reductions in preload or afterload. Cardiac output in the ejecting heart is a combination of coronary flow with aortic flow. For this reason, compared to a Langendorff preparation, it is very important that ejecting heart aortic cannulas are as close to the inner aortic diameter as possible.

Ejecting heart cardiac output (CO) is equal to the venous return from the lungs (represented by the oxygenator and heater in the circuit) to the left atrium (LA). The venous return is represented by the flow of perfusate from preload chamber via the atrial cannula. The LA perfusion line must be capable of delivering perfusate at a rate sufficient to support the maximum CO of a working heart at any particular preload. If the LA perfusion line is too small or there is an obstruction or debris decreasing flow and thus preload, it will falsely limit the CO. Using an inline flowsensor or in-line pressure sensor enables control of this parameter. Moreover, using a pressure or flow sensor ensures that LV filling is not limited by inadequate LA atrial inflow.

SENSOR	PARAMETERS MEASURED
PV transducer in LV	LVP, LVV, load dependent and load-independent properties
Aortic pressure transducer	aortic pressure
Aortic flow sensor	aortic flow
Atrial pressure transducer	preload pressure
Preload flow sensor	atrial inflow



Working heart set-up with pressure and flow sensors.

a. opening of the right coronary artery
b. opening of the left coronary artery
1. **Right semilunar cusp**
2. **Posterior semilunar cusp**
3. **Left semilunar cusp**
4. **Ascending Aorta**
5. **Suture location (in the groove)**
6. **Mitral valve**
7. **Left Pulmonary veins**
8. **Pressure catheter inserted into Tuohy Borst to monitor preload pressure**
9. **Pressure/PV catheter measuring cardiac load dependent and independent cardiac function (after temporary clamping of the preload line)**
10. **Tubing flowsensor placed on preload line. Tubing flowsensor placed on afterload line (not shown)**

transonic
THE MEASURE OF BETTER RESULTS.

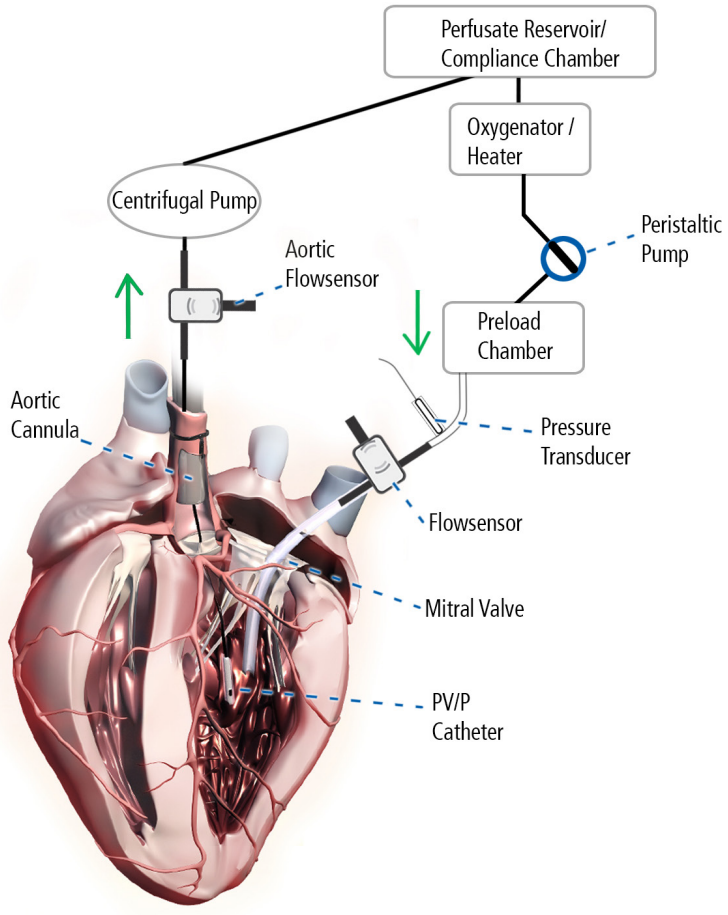
Working Heart Model for Cardiac Assessment Cont.

Filling of the LA can be determined by running the equipment without a heart attached and measuring the flow from the LA line. Flow rate of at least 150 ml/ min is recommended for a 1g heart. The perfusion fluid enters via the mitral valve into the left ventricle and from there it is ejected through the aortic cannula against a hydrostatic pressure set via the compliance loop. The afterload is determined by the height of the compliance reservoir above the aortic cannula. The compliance bubble trap contains a 2 mm diameter air bubble and mimics normal vascular elasticity. It is an essential component of the perfusion circuit and greatly increasing the chances of successful working heart function. In the course of left ventricular ejection, a portion of the perfusion fluid is forced into the coronary ostia and thereby perfuses the coronary vessels of the heart. Cardiac output from the IEH is detected and measured by the flowsensor. See schematic picture for more circuit details.

APPLICATIONS OF WORKING HEART SET-UP USING PV MEASUREMENTS

Steady-state relationships can be obtained by varying the loading conditions of the heart over a wide range preload and afterload to obtain:

- Investigation of positive and negative inotropic effect (pharmacology) including load independent values
- Calcium antagonism
- Metabolic studies
- Arrhythmogenic, anti-arrhythmic, anti-fibrillatory effects (pharmacology)
- Electrophysiological evaluation (mapping) using cardiovascular agents
- Fluorescent/luminescent imaging



Schematic representation of a set up of working heart model including possibilities for pressure, PV and flow measurements for ensuring control over the cardiac preparation. Please note: during the left ventricular ejection phase, a portion of the perfusate is forced into the coronary ostia enabling nutrient and oxygen rich perfusate to supply coronary vessels of the heart.

Pulse Wave Velocity (PWV)

During contraction the heart muscle creates a pressure wave which drives the blood out of the ventricle and through the vasculature. The pulse wave velocity (PWV) is the distance traveled by the pressure wave divided by the time for the wave to travel that distance.

$$PWV = \frac{\Delta x}{\Delta t}$$

The speed at which the pressure wave travels through the vasculature is dependent on the material properties of the vessels. Major arteries can be characterized as having viscoelastic material properties. Energy provided to a viscoelastic material during loading is both dissipated during unloading (viscous) and returned (elastic). The elastic return of energy creates reflected waves that travel back towards the heart. The relationship between the PWV and the stiffness of the vasculature can be expressed via the Moens-Korteweg equation, assuming that the artery wall is isotropic and experiences isovolumetric change with pulse pressure:

$$PWV = \sqrt{\frac{E \cdot h}{2r\rho}}$$

E = incremental elastic modulus
h = wall thickness
r = vessel radius
ρ = blood density

Fibroblast, vascular smooth muscle cells (VSMC), intermediate cells, pericytes, myocytes, mast cells, endothelium, nerves, interstitial macrophages are all present in the vascular wall. The principally important vascular viscosity players are fibroblast and VSMC, as they produce the extracellular matrix (ECM). The principle ECM components which impact PWV are elastin and collagen (types I and III). Inherent PWV increases with distance away from the heart due to decreased elastin and increased collagen content. Collagen content often increases during various disease states due to remodeling which in turn increases the pulse wave velocity.

When vascular stiffening occurs a higher pulse wave velocity is observed. Increased aortic stiffness can also alter the pressure wave's shape due to decreased capacitative properties of aortic vessel wall. During the systolic phase the aortic wall expands to accommodate the increased blood volume. Due to lower stiffness, the elastic portion of the vessel wall dilates at the beginning. When the wall strain increases, the vascular elastic modulus increases because the collagen fibers begin engaging in order to maintain the aortic wall shape (3).

PHYSIOLOGICAL FACTORS THAT IMPACT PULSE WAVE VELOCITY

PWV has to be assessed and closely monitored within the context of heart rate (HR) and mean arterial pressure (MAP). If MAP increases, arterial stiffness increases and PWV increases due to the exhaustion of elastin-limits and collagen engagement. The nervous system has the ability to regulate vascular tone and thus PWV.

SOME COMMON APPLICATIONS OF PULSE WAVE VELOCITY

- Measure regional elasticity of a vessel to predict the innate vessel properties, amount of remodeling, or stage of vascular disease.
- Compare regional physiological inherent vascular properties (e.g. PA vs. Ao).
- Assess phenotypic inter-species differences, or stage of vasculature change post-genetic (KO, KI, transgenic).
- Assess resistant hypertension and its treatment (persistently elevated and isolated systolic hypertension due to excess in aldosterone and an increased intravascular volume). Measure PWV to evaluate success of renal sympathetic denervation in the treatment of resistant hypertension.
- Assess the effect of sympathetic mediators based on changes in PWV in the aorta.

Pulse Wave Velocity (PWV) Cont.

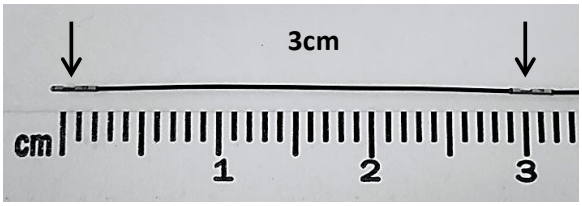
EXAMPLE METHOD: IN-VIVO MOUSE MODEL

The hallmark of vascular aging is vascular stiffening, predominantly due to loss of elastin and elevated collagen deposition. Pulse wave velocity measurements are the gold standard for vascular aging measurements in humans. Although vessel stiffening is challenging to measure in mice, it is for sure possible and should be measured with that same gold standard.

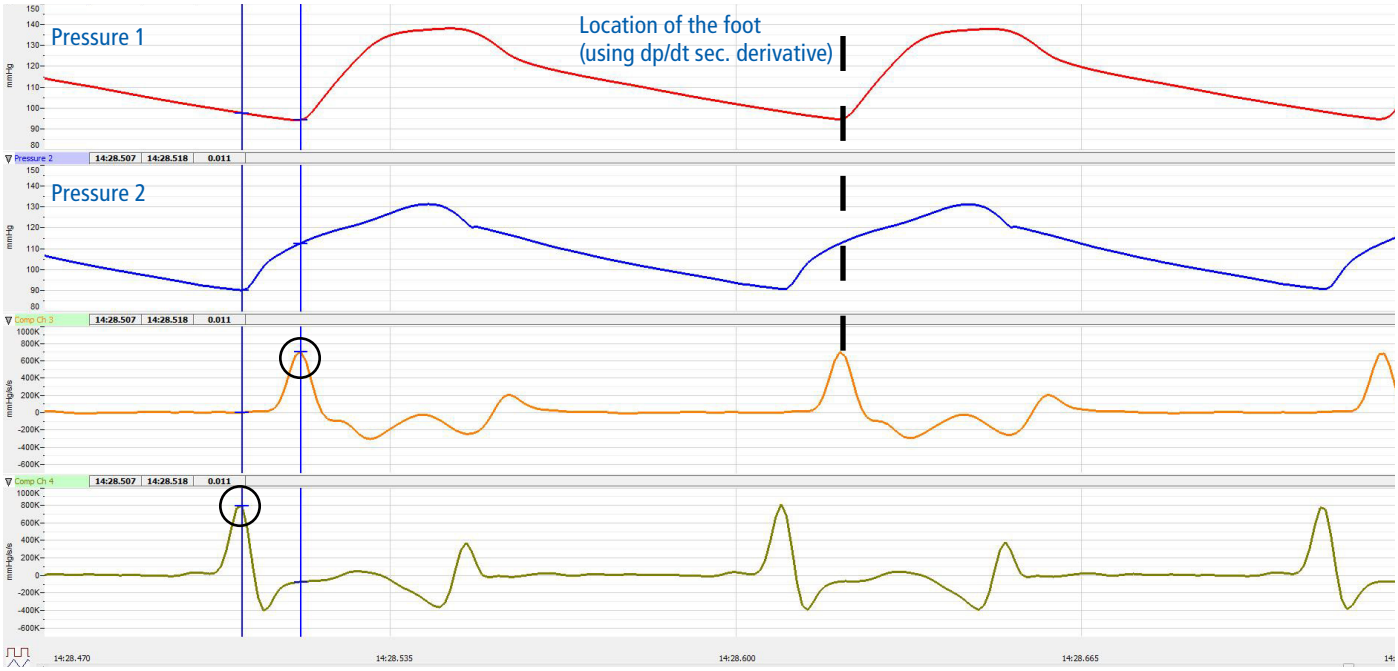
In the example below a dual sensor pressure catheter was inserted into the aorta via the left femoral artery, with simultaneous recordings taken from the descending thoracic aorta (proximal sensor) and the abdominal aorta (distal sensor). The location of the foot of the arterial pulse wave is determined using the second derivative maximum method in order to calculate the pulse transit time (PTT) which is the time it takes for the pressure signal to travel from one pressure sensor to the second.

The Pulse Wave Velocity can then be calculated:

$$PWV = \frac{PWD}{PTT} = \frac{0.03\ m}{0.01\ s} = 2.73\ m/s$$



1.2F double pressure catheter with known distance (3 cm) between pressure sensors. This distance is known as the pulse wave distance (PWD).



The second derivative maximum is used to locate the foot of the pressure pulse wave in order to calculate the pulse transit time (PTT) between the two sensors.

Mouse details: Sex: M Age: 10wk BW: 29.8g HR: 587 BPM

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Mouse Ascending Aorta and Left Ventricle Acute Pressure Measurement (Closed Chest Approach)

APPLICATION BASICS	
Site:	Left Ventricle - Closed Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute
CATHETER	
Size:	1.2F
Type:	Pressure
Catalog #:	FTH-1211B-0018
SYSTEM	SP200 or SP430

Application

The hemodynamic properties in the left ventricle measured by a pressure catheter can be used to gain insight in cardiac function.

Anatomical Landmarks

Right Carotid Artery (RCA) passes cranially along the right side of the trachea near the larynx in the close proximity to the vago-sympathetic trunk. Major muscles (sternohyoid and strenomastoid) in the area have to be moved aside to allow ventral neck access.

Pre-Surgical Preparations and General Anesthesia

Prepare an area for scrubbing in a separate location from where the surgical operation will take place. For cardiac surgery, it is best to find low-traffic area. Ideally, clean surfaces using disinfectants with low reaction to organic materials (e.g. Phenolics -- Lysol, TBQ).

Basic surgical supplies for mouse cardiac surgery should include a sterile surgical instrument pack and sterile supplies (i.e. drapes, gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask and sterile suture packs). In addition, a glass bead sterilizer, heating water blanket or approved electrical heating/feedback control unit should be used. Heat lamps are not ideal for body temperature maintenance and can often be a source of electrical noise/interference. Delicate rodent surgical instruments should be inspected for damage before sterilizing.

Set up surgical microscope (interpupillary distance, check light bulbs, adjust to check magnifications), organize surgical table and fine-tune surgical stool to a comfortable setting where the triangular position can be reached (both feet touching the ground with both arms comfortably resting on the surgical table). Turn on glass bead sterilizer.

Prepare 0.9% saline or a similar isotonic fluid and pre-warm the solution if it will be given pre-operatively. When a decision is made to use pre-warmed sterile isotonic fluids subcutaneously it is also suggested to use a preventive analgesia.

Before inducing anesthesia be sure to record weight, age, sex, strain, colony history and health status of each mouse, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check mouse's respiratory rate (80-240 breaths/min), heart rate (500-600 beats/min) and temperature (37.1-37.5°C).

Shave the animal while on the warming pad using ChronMini cordless clippers. Remove any remaining hair from the surgical area using a depilatory cream (e.g. Nair). Apply surgical scrub alternating between disinfectant (i.e. iodophores, chlorhexidines) and alcohol. Please remember: Iodophores will inactivate a wide range of microbes, however literature describes their reduced activity in the presence of organic matter.

Use gauze squares for scrubbing. Scrubbing should always begin along the incision line and extend outwards, ensuring contaminants are not pulled towards the surgical site. Always scrub larger surface area then surgical field. Do not wet large area of skin or fur with alcohol to avoid hypothermia. Consider using drapes to maintain a sterile field and preserve body temperature.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse LV Acute Pressure Measurement (Closed Chest) Cont.

Pre-Surgical Preparations and General Anesthesia Cont.

Pre-anesthetize mouse for cardiac surgery with 3-4% Isoflurane (Forane) mixed with driving gas (Oxygen) 0.5 L/min inhaled in Plexiglas induction chamber with lid. It is important not to disturb mouse during induction. Apply an ophthalmic ointment to both eyes following induction of anesthesia to prevent corneal drying.

Use pre-cut Styrofoam as a reclined platform with rubber band attached to the edges at the top to allow mouse's neck to be situated at the top with rubber band attached to his upper incisors. Use atraumatic forceps to carefully pull out the tongue. Trans orally intubate using a 22-gauge polyethylene catheter with help of fiberscope by directly illuminating ventral area of the neck. Insert catheter into the larynx past the 2 valves (vocal cords). Ventilate with tidal volume of 0.2 mL, with 128 ventilation cycles per minute. Keep the intubation catheter in alcohol between intubations for disinfection, use 50 mL syringe to clear off any residual alcohol, to avoid aspiration.

Once connected to the ventilator, inspect breathing pattern, color of membranes and capillary refill time. If feasible, use pulse oximetry. We have found that Isoflurane produces an excellent long-term controllable anesthesia for cardiac surgery. Adequate anesthesia is accompanied by loss of muscle tone and by loss of reflexes (e.g. corneal, pinnae and pedal).

Regulate post-induction anesthesia to 2% with animal placed on a warming pad (38°C) in a supine position, with the upper and lower extremities attached to the table with surgical tape. Maintain mouse on 2% Isoflurane by using rodent ventilator operated in volume-controlled mode with a maximal stroke volume from 30-350 µL, and a positive-end expiratory pressure of 1–3 cm H2O. Prior to surgery calculate the ventilator set up. Formula is based on animal mass (Mb):

- Respiration rate (RR, min^{-1}) = 53.5 * $M_b^{-0.26}$
- Tidal volume (V_t, ml) = 6.2 * $M_b^{1.01}$

It is recommended that a "circle re-breathing circuit" with the vaporizer positioned outside of this system is used for anaesthetic delivery. Control successful ventilation by running blood gas analysis to confirm normal gas exchange.

Prior to surgery, soak the tip of the pressure catheter in 0.9% saline for ~ 20 minutes. Connect the SP200 system to the data acquisition software, ensuring that the software channel is calibrated. See Manual and Quick Start Guide for more details. After soaking, adjust the pressure balance to zero for atmospheric pressure.

Mouse Weight (g)	RR (min^{-1})	V_t (µl)
20	148	119
35	128	209
50	117	301

Other methods of anesthesia may be used. Be sure to consider cardiovascular impact of anesthetic choice. Please adhere to your institutions guidelines for anesthesia and pain management. See"Rodent Anesthesia Guidelines" on page 45 for more considerations.

Mouse LV Acute Pressure Measurement (Closed Chest) Cont.

Surgical Approach

For right common carotid artery (RCA) access, secure animal in supine position on the heating pad. Using sharp scissors, starting immediately below the chin, make a straight incision in a direction towards the transversal pectoral muscles. Make the incision as straight as possible while lifting the skin with thumb forceps (Fig 1). Keep the scissor tips up. Using blunt scissors or medium hemostats, blunt dissect any underlying glandular tissue from skin around the entire circumference of the wound (Fig 2). Minor bleeding can be stopped by Q-tips or by pre-made spear shaped nitrocellulose sponges (Harvard app, QC). Keep area moist with warm sterile saline or PBS. Gently separate glands via blunt dissection to expose underlying muscular layer and use retractors to make trachea and ventral neck muscle visible (Fig 3).

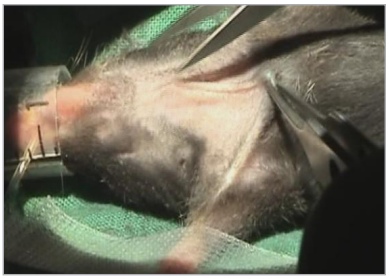


Fig. 1: Initial incision under the chin

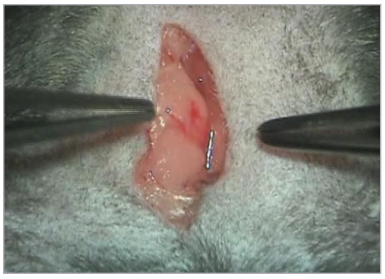


Fig. 2: Dissect glandular tissue from skin

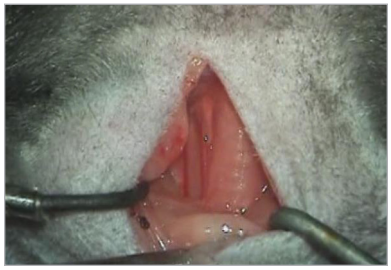


Fig. 3: Retract skin to expose site

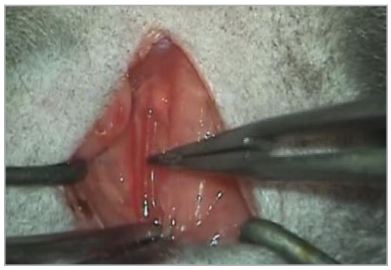


Fig. 4: Expose & dissect the right common carotid artery

Bluntly dissect along the longitudinal right central and adjacent muscular group (sternocleidomastoid, thyrohyoid, sternohyoid, omohyoid) and remember to avoid pressure on these muscles to maintain the rat's ability to breath. Carefully separate the central muscle from parallel neck muscles and the diagonal thin muscular band (omohyoid) lying directly over the carotid vasculature. Retract skin and muscular tissues for visualization of the underlying carotid artery (Fig 4). Keep the tips of the instruments up and all tissues moist and warm. During subsequent methodical dissection and retraction of adjacent tissue and sheets, RCA can be detected next to vago-sympathetic trunk (a thin white sheath lying next to the RCA).

Continue blunt dissection to expose RCA to about 20 mm in length. Dissect alongside the RCA distally towards the head to expose RCA's bifurcation into branches. Ensure that section of the RCA is completely separated from all adjacent tissues to limit an unexpected bleeding during the retraction and/or clamping procedures. RCA must be fully separated from vascular fascia and the vagus nerve.

At this stage 5-0 sutures can be placed around RCA to be used for retraction and/or clamping and hemostasis. Use micro-forceps to place sutures around the RCA (Fig 5). Place the first suture to the most proximal visible end on the RCA (as close to the head as possible) and tie it off using surgical knot (Fig 6), while creating tension with a clamp and retract it towards the head. Place 2nd suture (Fig 7) and retract distally towards the tail. At this point the RCA has been retracted proximally and distally and blood flow has been temporally stopped. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry.

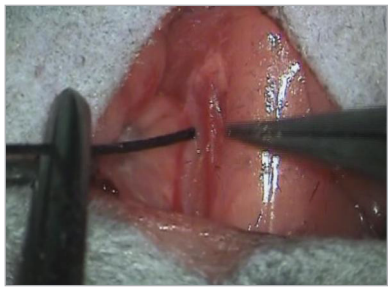


Fig. 5: Use hemostat to draw suture under the RCA

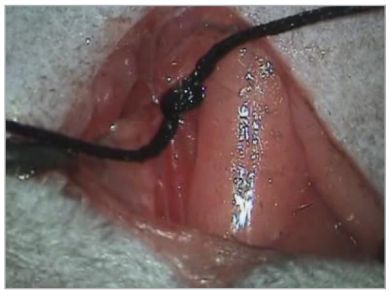


Fig. 6: Tie suture to proximal end of RCA

Mouse LV Acute Pressure Measurement (Closed Chest) Cont.

Surgical Approach Cont.

Slide 3rd suture under the segment but do not tie it off (Fig 7). This suture will be tied off when the pressure catheter passes the second suture on the way into the aorta and heart. While creating tension on the distally placed sternal-suture, make a cut with micro-dissecting scissors closer to the head (proximally on the free RCA segment) (Fig 8). Keep in mind that a longer isolated section of the RCA will significantly improve chances for successful Catheter introduction.

Following a successful RCA arteriotomy use a vascular introducer as described in Rat RCA catheterization or micro forceps (Fig 9) to open and lift the incision, while exploring the size of this opening. Especially for a novice surgeon, who might take more time to successfully introduce the Catheter, an introducer might allow more time for location of the insertion in the collapsed RCA, limiting blood loss on catheterization. When completely satisfied with RCA opening carefully proceed (Fig 10) and lift the sternal clamp and insert 1.2F pressure catheter into the opening. Position and tie off the first suture around the catheter about 1 cm past the pressure sensor (Fig 11). At the same time, please make sure there is not an excessive resistance present upon introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy incision on repositioning(s).

With the catheter in the RCA, get a feel for the degree of resistance while gently rotating the catheter in the RCA. Slide the catheter slowly towards the heart. Then tie off the second 5-0 suture around the catheter to prevent slip out (Fig 12). Be careful not to damage the catheter with the forceps tips, and be sure to hold the catheter in the same plane as the blood vessel during the entire introduction process. Slowly introduce the catheter further until you see the aortic pressure trace (see next page).

When the catheter is inserted further through the aortic valve into the left ventricle, the aortic pressure trace will transition to the LV pressure waveform. Allow Catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol.

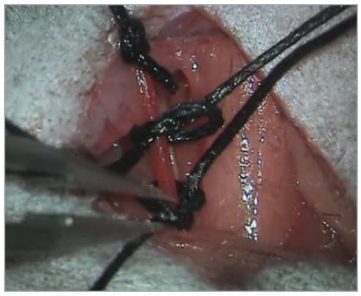


Fig. 7: Three sutures around the RCA

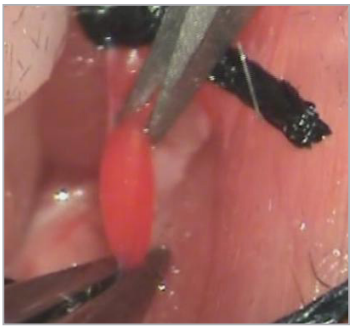


Fig. 8: Carefully cut RCA

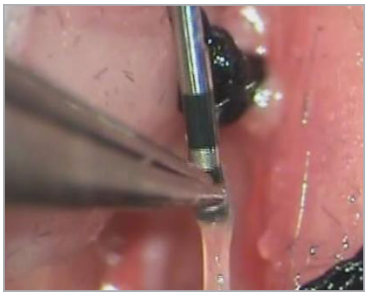


Fig. 9: Carefully insert Catheter

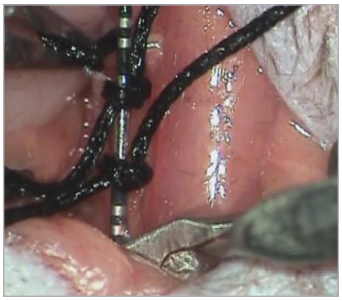


Fig. 10: Proceed with Catheter insertion

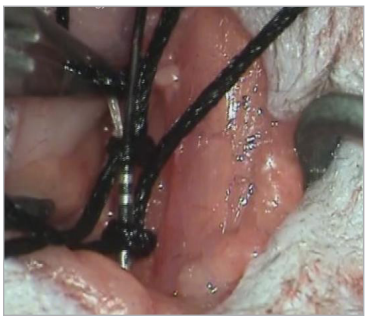


Fig. 11: Fully insert both sets of volume rings past the sutures

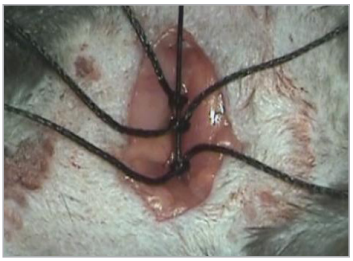
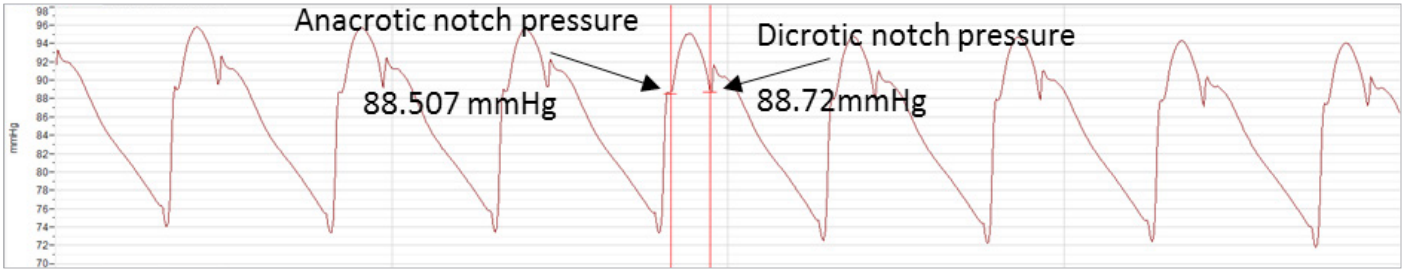


Fig. 12: Secure Catheter in place

ACKNOWLEDGMENTS

Cardiovascular Division, British Heart Foundation Excellence Centre, King's College London, St Thomas' Hospital, London SE1 7EH, UK

Mouse Acute Aortic Pressure Measurement (Closed Chest) Cont.



Example of acute mouse aortic pressure with anacrotic and dicrotic notches marked.

Aortic Pressure

The dicrotic notch on the aortic pressure waveform indicates the completion of systole and represents closure of the aortic valve and subsequent retrograde blood flow. Anacrotic notch on the aortic pressure waveform indicates drop of pressure during its development (from diastole through dpmax) to peak systolic pressure. The anacrotic notch in central (aortic) pressure is physiologically present. On the other hand, if it is present at carotid artery pulse pressure then aortic stenosis might be present (1).

REFERENCES

(1) Textbook of Cardiovascular Medicine, Third ed. Pages 197-198; Lippincott Williams & Wilkins, by Eric J. Topol MD and Robert M. Califf MD

(2) The influence of heart rate on augmentation index and central arterial pressure in humans. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. J Physiol. 2000 May 15;525 Pt 1:263-70.

INVASIVE CENTRAL AORTIC PRESSURE DETERMINATION OF PP, DELTA P AND AI

Delta P is the change of pressure between the inflection point to the systolic pressure.

In this example Delta P= 6.58 mmHg

Pulse Pressure (PP) is in central pressure from diastolic to P max (systolic pressure).

In this example PP= 21.72 mmHg

Augmentation Index (AI) = delta P/ PP in (%).

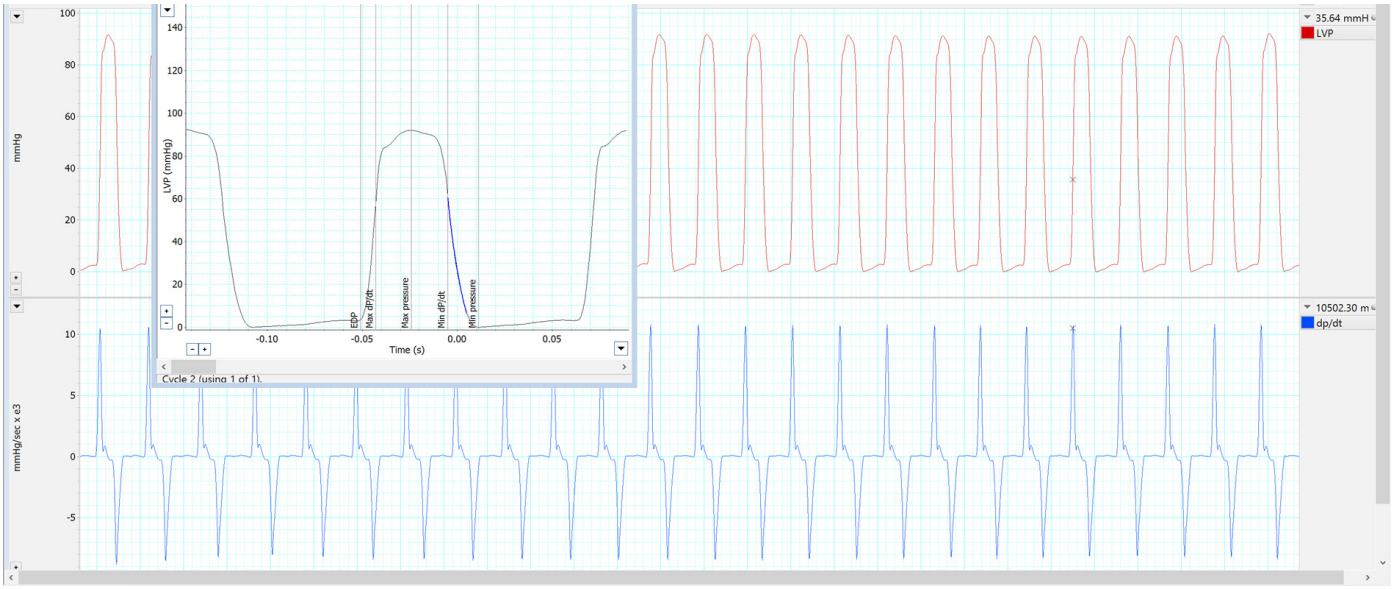
In this mouse example AI is:

AI = 6.58 mmHg / 21.72 mmHg = 0.3 = 30%

Invasive central pressure Augmentation Index (iAI), is characterized in this example as pressure difference (delta P) between the inflection on the aortic pressure wave before Pmax (max systolic pressure) that expressed as a ratio of pulse pressure (PP).

As the HR increases the AI is decreasing (linearly). Possibly due to alterations in the timing of the reflected pressure wave, produced by changes in the absolute duration of systole (1).

Mouse LV Acute Pressure Measurement (Closed Chest) Cont.



Example of recording of acute mouse Left Ventricle (LV) pressure. Channels are as follows: Ch1 raw LVP, Ch2 LV dp/dt.

Mouse was intubated and anesthesia performed by ventilation using MiniVent type 845 (Hugo Sachs, Harvard Apparatus), using 100% oxygen with 1% of isoflurane.

	HR bmp	P_{MAX} mmHg	P_{MIN} mmHg	EDP mmHg	MEAN PRESSURE mmHg	SYSTOLIC DURATION sec	DIASTOLIC DURATION sec	CYCLE DURATION sec	dP_{MAX} mmHg/sec	dP_{MIN} mmHg/sec	TAU Sec
LVP MEAN	539.45	91.57	0.08	3.31	34.00	0.046	0.065	0.111	11600.10	-9468.08	0.009

Mouse Right Ventricle Acute Pressure Measurement (Closed Chest Approach)

APPLICATION BASICS	
Site:	Right Ventricle - Closed Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute
CATHETER	
Size:	1.2F
Type:	Pressure
Catalog #:	FTH-1211B-0018
SYSTEM	SP200 or SP430

Application

The hemodynamic properties measured by the pressure catheter can be used to determine cardiac function. Right ventricular pressure is often measured as part of pulmonary disease related research questions.

Anatomical Landmarks

Closed chest approach for right ventricle (RV) catheterization is through the right common jugular vein (RJV). To access the RV without opening the chest cavity, the RJV is superior as compared to the left jugular vein as it is associated with a more direct and easier access the right atrium and sinus venosus for passing the Catheter into its final destination in the RV cavity. Anatomically there are two sets of jugular veins: external and internal. The left and right external jugular veins (LEJV and REJV) drain into the subclavian veins. The internal jugular veins (RIJV and LIJV) join with the subclavian veins more medially to form the brachiocephalic veins. Finally, both brachiocephalic veins join to form the superior vena cava (SVC) that enters the right atrium. For purpose of this application note RJV is considered joined area of both RIJV and REJV.

Pre-Surgical Preparations and General Anesthesia

Prepare an area for scrubbing in a separate location from where the surgical operation will take place. For cardiac surgery, it is best to find a low-traffic area. Ideally, clean surfaces using disinfectants with low reaction to organic materials (e.g. Phenolics -- Lysol, TBQ).

Basic surgical supplies for mouse cardiac surgery should include a sterile surgical instrument pack and sterile supplies (i.e. drapes, gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask and sterile suture packs). In addition, a glass bead sterilizer, heating water blanket or approved electrical heating/feedback control unit should be used. Heat lamps are not ideal for body temperature maintenance and can often be a source of electrical noise/interference. Delicate rodent surgical instruments should be inspected for damage before sterilizing.

Set up the surgical microscope (interpupillary distance, check light bulbs, adjust to check magnifications), organize the surgical table and fine-tune the surgical stool to a comfortable setting where the triangular position can be reached (both feet touching the ground with both arms comfortably resting on the surgical table). Turn on the glass bead sterilizer.

Prepare 0.9% saline or a similar isotonic fluid and pre-warm the solution if it will be given pre-operatively. When a decision is made to use pre-warmed sterile isotonic fluids subcutaneously it is also suggested to use a preventive analgesia.

Before inducing anesthesia be sure to record weight, age, sex, strain, colony history and health status of each mouse, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check mouse's respiratory rate (80-240 breaths/min), heart rate (500-600 beats/min) and temperature (37.1-37.5°C).

Shave the animal while on the warming pad using ChroMini cordless clippers or similar. Remove any remaining hair from the surgical area using a depilatory cream (e.g. Nair). Apply surgical scrub alternating between disinfectant (i.e. iodophores, chlorhexidines) and alcohol. Please remember: Iodophores will inactivate a wide range of microbes,

Mouse RV Acute Pressure Measurement (Closed Chest) Cont.

however literature describes their reduced activity in the presence of organic matter.

Use gauze squares for scrubbing. Scrubbing should always begin along the incision line and extend outwards, ensuring contaminants are not pulled towards the surgical site. Always scrub a larger surface area then the surgical field. Do not wet a large area of skin or fur with alcohol to avoid hypothermia. Consider using drapes to maintain a sterile field and preserve body temperature.

Pre-anaesthetize mouse for cardiac surgery with 3-4% Isoflurane (Forane) mixed with driving gas (Oxygen) 0.5 L/min inhaled in plexiglas induction chamber with lid. It is important not to disturb the mouse during induction. Apply an ophthalmic ointment to both eyes following induction of anesthesia to prevent corneal drying.

Later there are two options to maintain the Isoflurane anesthesia for this type of catheter insertion. One is to intubate the animal using the atraumatic technique to trans orally intubate using a 22-gauge polyethylene catheter and the other is to use a nose cone connected to semi-open or semi-closed circuits. Both techniques are suitable for this type if procedure, however intubation is associated with higher survival rate, especially for novice surgeons. For the intubation technique description please refer to the close or open chest mouse LV pressure volume catheterization protocols.

When connected to a breathing circuit, inspect breathing pattern, color of membranes and capillary refill time. If feasible, use pulse oximetry. We have found that Isoflurane produces an excellent long-term controllable anesthesia for cardiac surgery. Adequate anesthesia is accompanied by loss of muscle tone and by loss of reflexes (e.g. corneal, pinnae and pedal).

Regulate post-induction anesthesia to 2% with animal placed on a warming pad (38°C) in a supine position, with the upper and lower extremities attached to the table with surgical tape. Maintain mouse on 2% Isoflurane by using rodent ventilator operated in volume-controlled mode with a maximal stroke volume from 30-350 µL, and a positive-end expiratory pressure of 1–3 cm H2O. Prior to surgery calculate the ventilator set up. Formula is based on animal mass (Mb):

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Mouse Weight (g)	RR (min^{-1})	V_t (µl)
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Other methods of anesthesia may be used. Be sure to consider cardiovascular impact of anesthetic choice. Please adhere to your institutions guidelines for anesthesia and pain management. See”Rodent Anesthesia Guidelines” on page 45 for more considerations.

Surgical Approach

For right jugular vein (RJV) access, secure animal in supine position on the heating pad. Using sharp scissors, starting immediately below the chin, make a straight incision in a direction towards the transversal pectoral muscles. For access location please see Fig. 1. Make the incision as straight as possible while lifting the skin with thumb forceps (Fig 2). Keep the scissor tips up. Use blunt scissors, medium curved forceps, or hemostats to blunt dissect any underlying glandular tissue from skin around the entire circumference of the wound (Fig 3). Minor bleeding can be stopped by Q-tips or by pre-made spear shaped nitrocellulose sponges (Harvard app, QC). Keep area moist with warm sterile saline or PBS.

Mouse RV Acute Pressure Measurement (Closed Chest) Cont.

Gently separate glands via blunt dissection to expose underlying muscular layer and use retractors to make the RJV visible (Fig 4).

At this stage use micro-forceps to place 6-0 silk sutures around the separated RJV. The common RJV is not usually apparent unless the branches of external or internal JV are moved aside. Place the first suture to the most cranial end of the exposed RJV and tie it off using a surgical knot (Fig 5) to limit unexpected bleeding during the retraction and/or clamping procedures. Create tension with clamp and retract suture towards the head. Place a second silk 6-0 suture, and do not ligate but retract caudally towards the tail. At this point the RJV has been retracted cranially and caudally while blood flow has been temporarily stopped. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. Keep in mind that a longer isolated section of the RJV will significantly improve chances for successful catheter introduction.

While creating tension on the (non-tied) caudal-suture, make a cut with micro-dissecting scissors on the free and visible cranial portion of the RJV segment and position the catheter towards the venotomy opening (Fig 6).

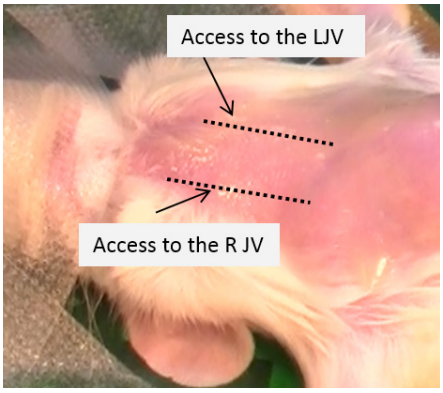

Fig. 1: Location of access to the RJV

Fig. 2: Initial incision under the chin

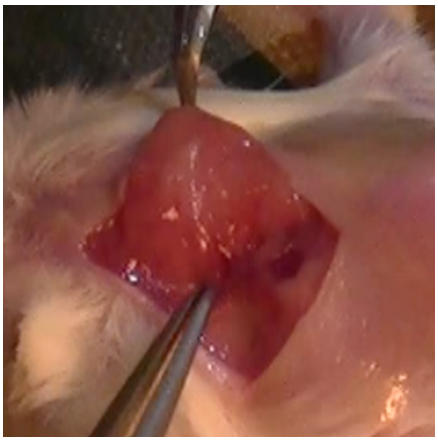

Fig. 3: Retract skin to expose site

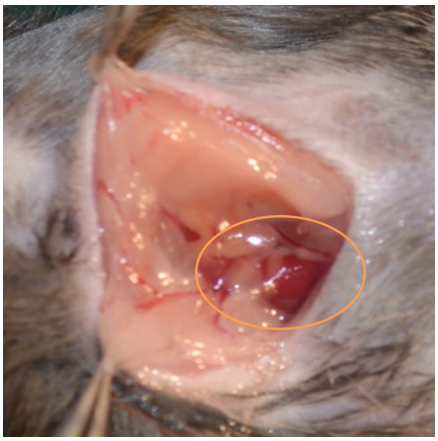

Fig. 4: Expose & dissect RJV

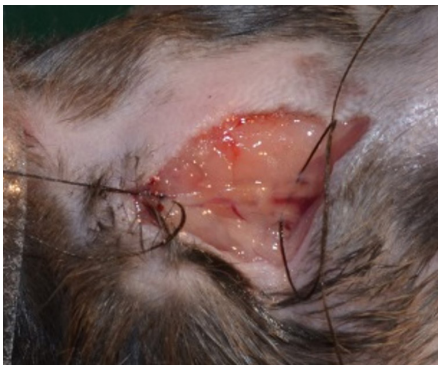

Fig. 5: Tie suture to the cranial end of the exposed RJV. Loosely prepare suture at the caudal end.

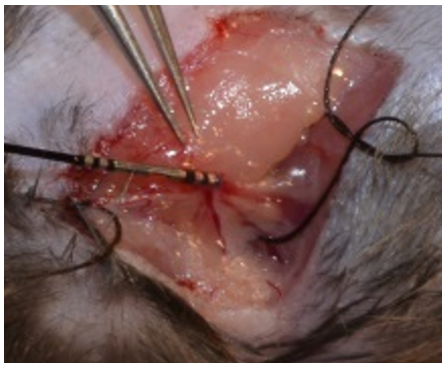

Fig. 6: Position Catheter parallel to the RJV

Mouse RV Acute Pressure Measurement (Closed Chest) Cont.

CATHETER INTRODUCTION AND POSITIONING

Following a successful RJV venotomy use micro or jewel forceps with tips covered by PE50 tubing to grasp the long shaft of the pressure catheter and aim for the venotomy (Fig 7). Catheter has to be positioned along the long axis of the venous segment.

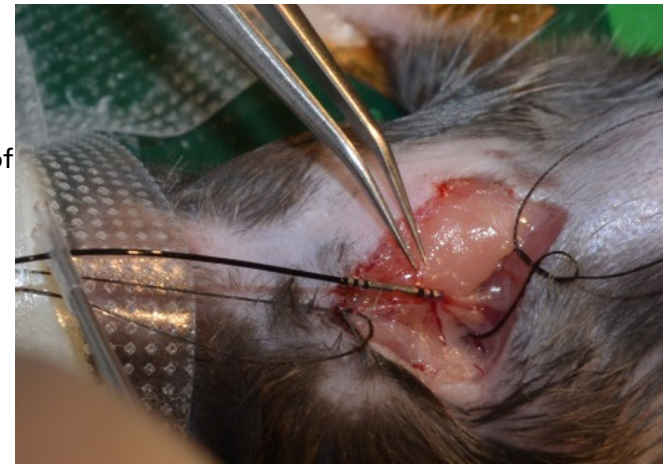

Fig. 7: Carefully insert the Catheter into the RJV

Please note: Vascular introducers can be used to open and lift the incision, while exploring the size of this opening. Especially for a novice surgeon, who might take more time to successfully introduce the catheter, an introducer might allow more time for location of the insertion in the collapsed RJV, limiting blood loss on catheterization. When completely satisfied with the RJV opening carefully proceed to insert the catheter with your dominant hand into the opening. Position and tie off the caudal suture around the catheter. At the same time, please make sure there is not excessive resistance present upon introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the incision on repositioning.

With the catheter in the RJV, get a feel for the degree of resistance by gently rotating the catheter. Slide the catheter slowly towards the heart. Then tie off a second 6-0 suture around the catheter to prevent slip out (optional, since the pressure in the vein is lower as compared to the right carotid artery). Be careful not to damage the catheter with the forceps tips and hold the catheter in the same plane as the blood vessel during whole introduction. Ideally there is very low amount of bleeding post insertion.

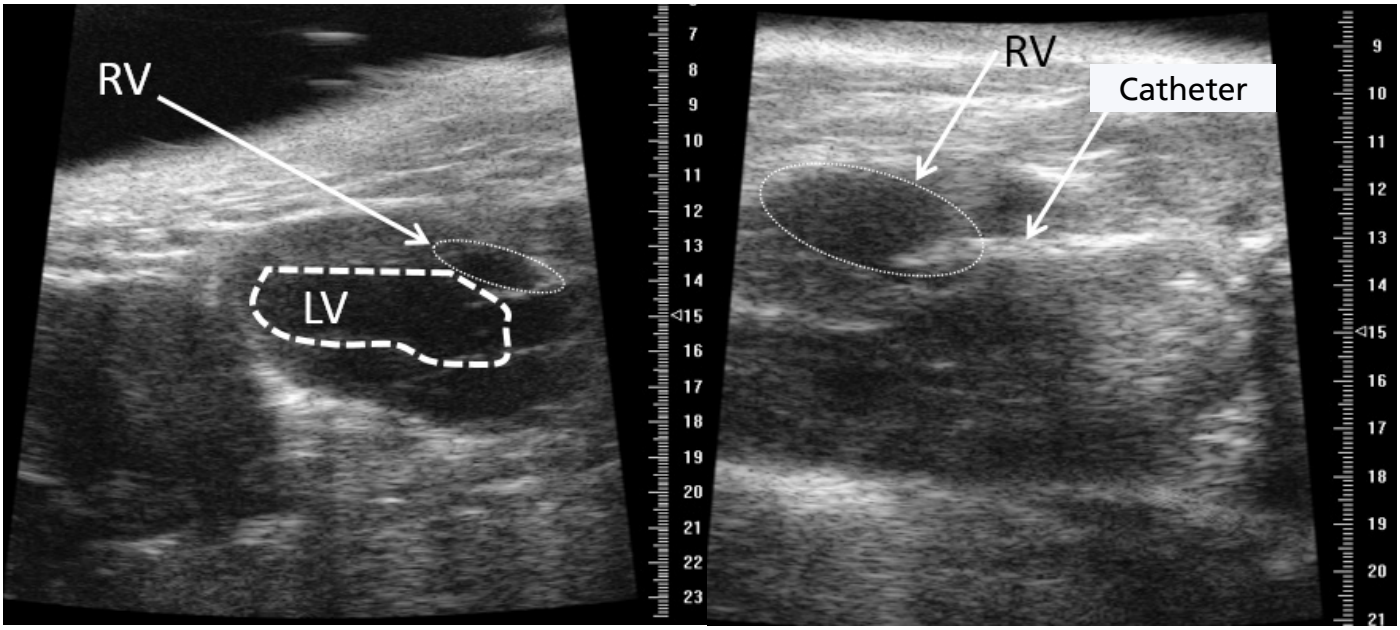

Fig. 8: (a) Long axis echocardiography image. LV and RV are encircled (b) The scan head is repositioned to better visualize the RV for catheter insertion.

Mouse RV Acute Pressure Measurement (Closed Chest) Cont.

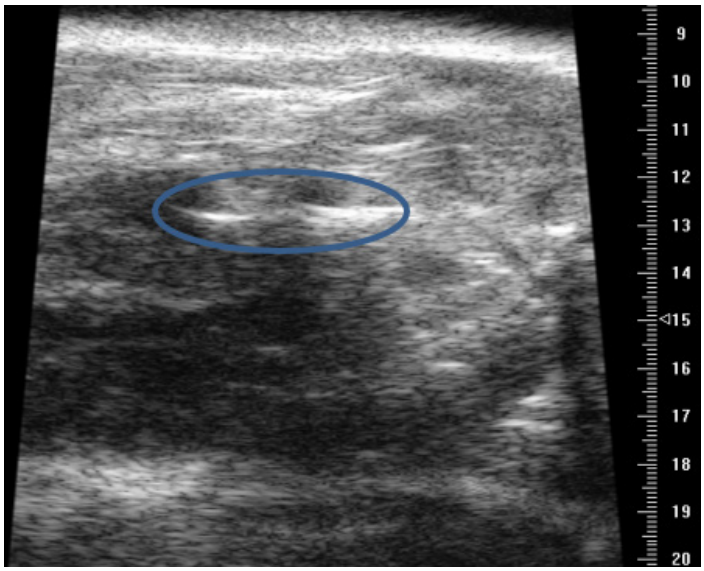

Fig. 9a: The blue circle shows the tip of the catheter advancing into position in the middle of the RV (seen at 2D plane)

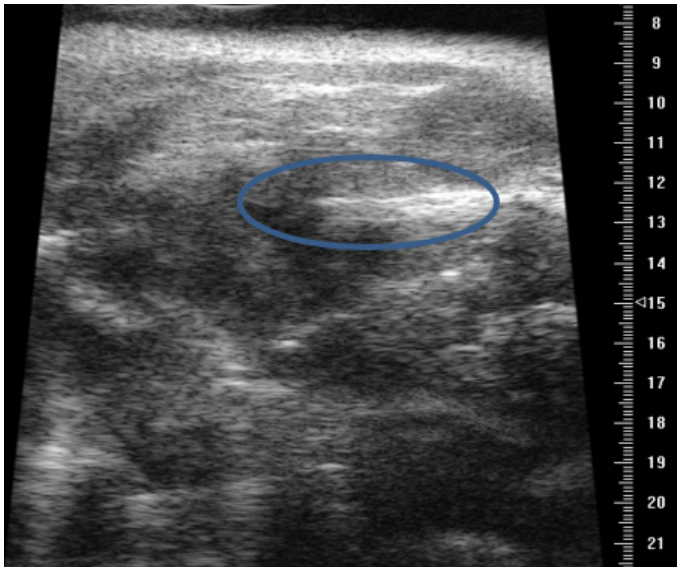

Fig. 9b: Make sure that the catheter (encircled) is not touching the septal or RV wall by constantly situating and updating the echocardiography scan head position on mouse's chest

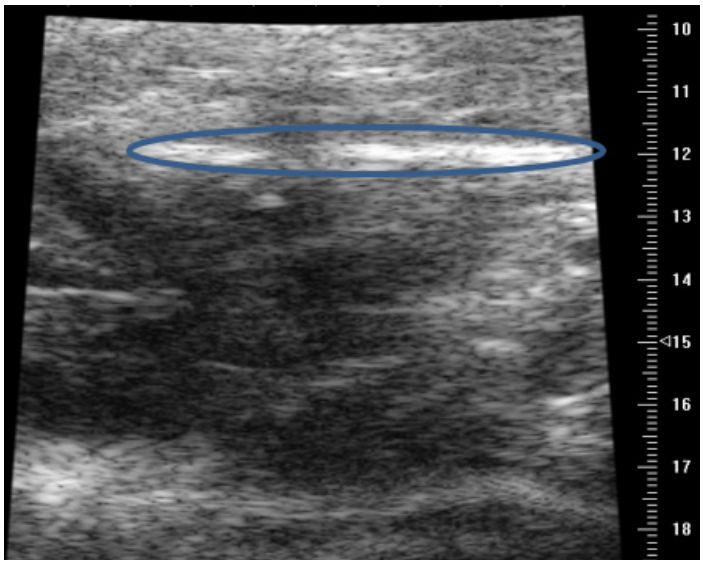

Fig. 10a: Catheter touching to the septum (blue circle)and as described it is also too far in the RV (long axis view)

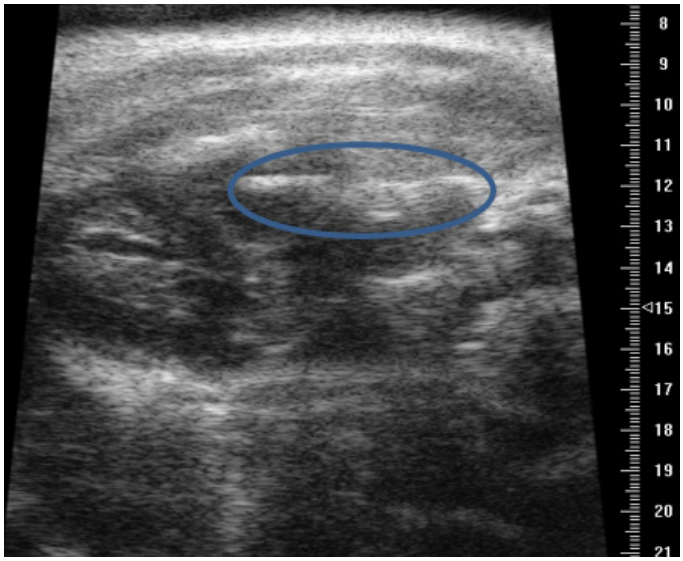

Fig. 10b: Catheter pulled back into better position. Both RV free wall and septum are away from the catheter at this point .This is a very good position for pressure recording in the RV.

Mouse RV Acute Pressure Measurement (Closed Chest) Cont.

CATHETER INTRODUCTION AND POSITIONING CONT.

For closed chest preparation it is wise to use echocardiography guidance to successfully position the pressure catheter into the RV. This procedure requires patience and concentration to overcome micromanipulation pitfalls that lead to unnecessary damage resulting in vascular penetrations and discrete bleeding into cavities (causing tamponade or hemothorax). When using echocardiography guidance first find long axis of the heart. Using the long axis find the RV position (Fig 8A). Position of the RV may vary, based on animal strain or shifting from surgery performed beforehand. Use cranial suture to reposition catheter towards the RV long axis. At this point, reposition the echo transducer to better visualize the RV (Fig 8B). Advance the catheter further into the RV cavity without touching the septal or RV free wall (Fig 9A, 9B). Observe the catheter position in the cavity before making pressure measurements (Fig 10A ,10B). When the catheter is in a good position, you will find good RV pressure signal (Fig 11). Allow the Catheter to stabilize in the RV for 5-10 min before marking the data file to start protocol.

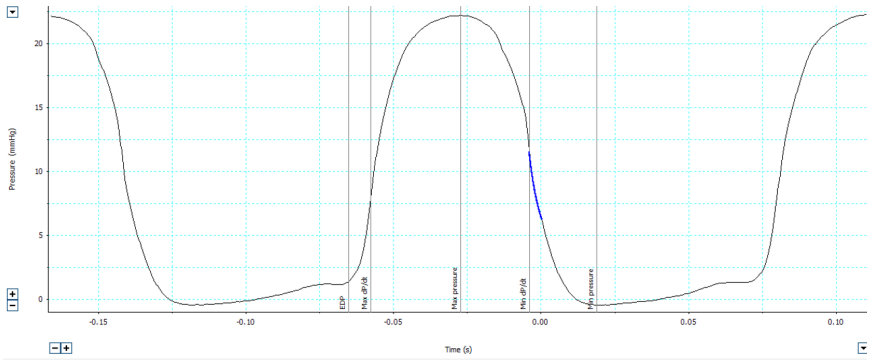

Fig. 11: Pressure signal from the mouse right ventricle.

ACKNOWLEDGMENTS

Transonic Scisense Inc. would like to thank Dr. Vahagn Ohanyan MD, PhD Research Assistant Professor of Integrative Medical Sciences, "Mouse microsurgery and echocardiography workshop" program director Northeast Ohio Medical University (NEOMED) 4209 State Route 44, Rootstown, OH 44272, United States for his help to create these echo-guiding images using VeVo 770, VisualSonics (Toronto, ON).

Mouse Acute Pulmonary Artery Pressure Measurement

APPLICATION BASICS

Site:	Pulmonary Artery -Open chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure
Catalog #:	FTH-1211B-0018

SYSTEM

SP200, SP430, ADV500

Application

Pulmonary artery hypertension (PAH) can be determined by direct measurement of pressure in the pulmonary artery (PA). Remodeling in the PA might influence the overall vascular properties of the artery. Performing measurements can help to assess overall health of the artery.

Anatomical Landmarks

Open chest approach - thorax/upper abdomen area over the xyphoid, proximity of the sternal manubrium. Cut through the diaphragm to expose the apex of the heart. To reduce bleeding avoid incisions around the sternum.

Surgical Approach

Secure animal in supine position on the heating pad. Make skin incision in the lower thorax/ upper abdomen area over the xyphoid (Fig 1). Separate the skin from the chest wall by blunt lateral dissections. Open the abdominal wall in the proximity of the sternal manubrium (Fig 2). Use 45 cm 5-0 softsilk on 3/8 circle 19 mm cutting needle to penetrate xiphoid and to pull and attach 5-0 suture proximally (Fig 3). Cut through the diaphragm (Fig 4) to expose the heart apex (Fig 5). Try to avoid any incisions around the sternum to limit bleeding from internal mammary arteries. Try not to artificially retract rib cage. Gently maneuver the apex, using Q-tips into the diaphragm opening. Using microinstruments, bluntly open pericardium (Fig 6).

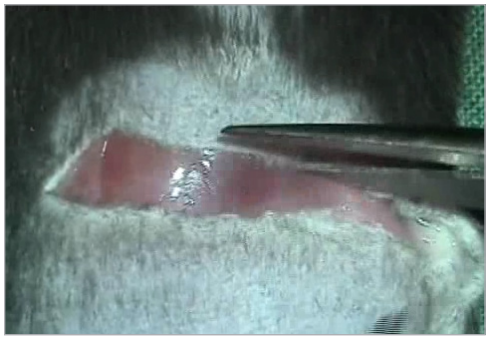

Fig. 1: Initial incision in the upper abdomen

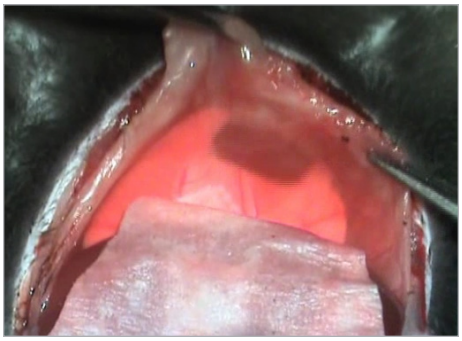

Fig. 2: Opening the abdomen wall

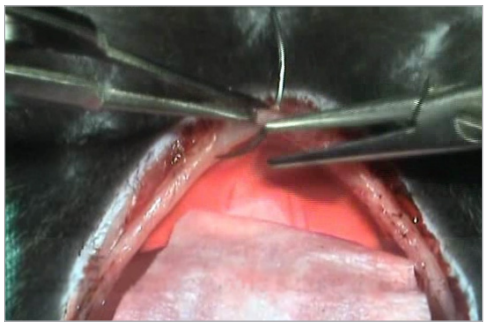

Fig. 3: Use a suture to hold the xiphoid in place

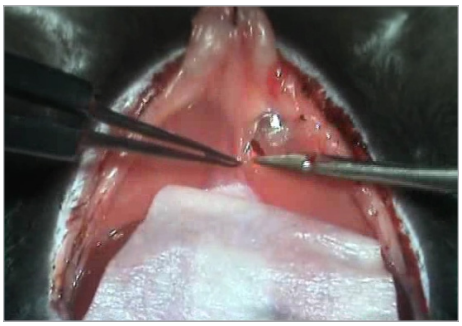

Fig. 4: Cut through the diaphragm

Use a 27G needle for the right ventricle (RV) apical stab. After a successful stab, blood is usually found in the needle conus. As the needle is withdrawn from the RV myocardium use a spear shaped Q-tip to help hold the RV down (Fig 7). The 1.2F pressure catheter is introduced through the stab wound, best done under magnification, with the dominant hand using forceps covered by PE tubing until the pressure sensor is fully surrounded by RV muscle (Fig 8).

ACKNOWLEDGMENTS

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Mouse Acute PA Pressure Measurement Cont.

Surgical Approach Cont.

Allow the catheter to stabilize in the RV for 2 minutes while collecting the RV pressure similar to Fig. 9, before marking the data in the file to start the protocol. Positional adjustment of the catheter needs to be made based on acquired pressure signal that will mark the transition into the pulmonary artery (PA) (Fig. 10). Carefully reposition the catheter until an optimal position is found (Fig. 11). Document PA pressure wave and make note in the recorded file.

At the end of the experiment, carefully remove the pressure catheter by gently pulling it back through the stab wound. Immediately, insert catheter tip into 5 ml saline pre-filled syringe. Clean catheter as soon as possible according to proper care guidelines to considerably prolong the catheter's life (Catheter Cleaning & Disinfecting Guide).

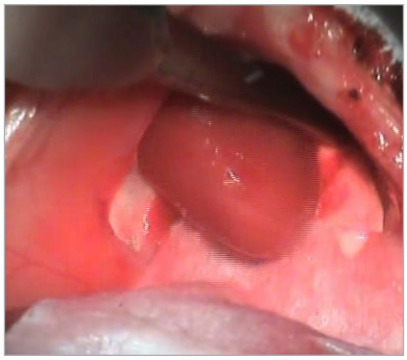


Fig. 5: Expose the heart apex

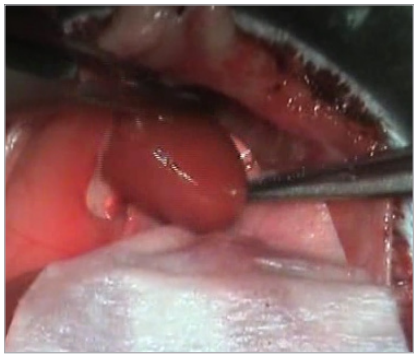


Fig. 6: Open the pericardium

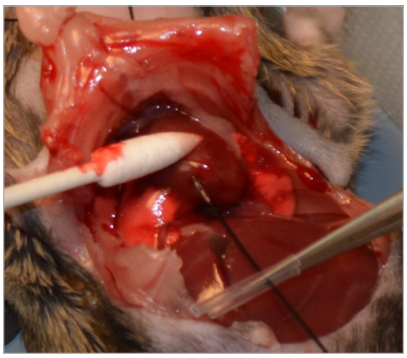


Fig. 7: Carefully insert the catheter into the RV through the apical stab

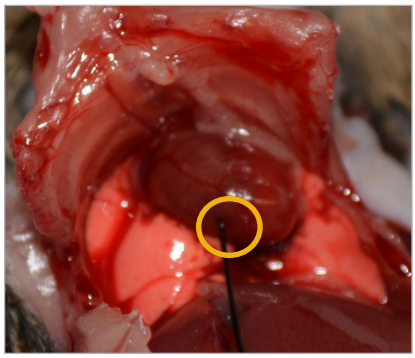


Fig. 8: Insert catheter until the pressure sensor is fully submerged. The yellow circle indicates the relative location of the PA.

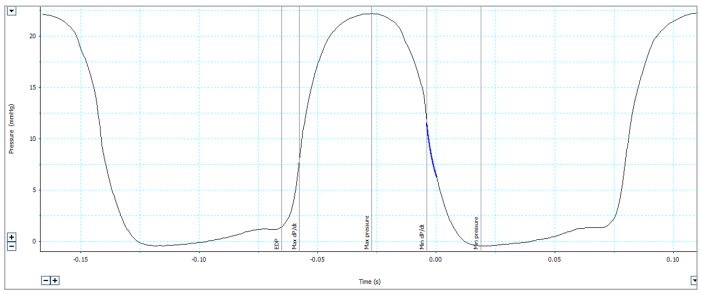


Fig. 9: Mouse right ventricle (RV) pressure

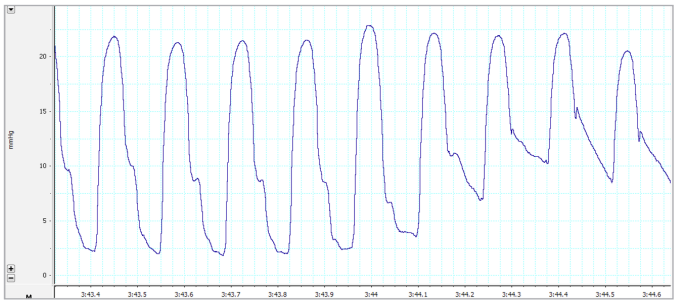


Fig. 10: Mouse pressure as the catheter transitions from the RV to the pulmonary artery (PA). Transition noted by the change in end diastolic pressure (EDP) and the appearance of the dicrotic notch

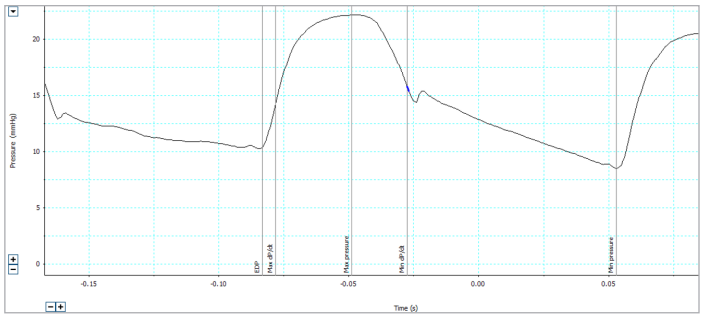
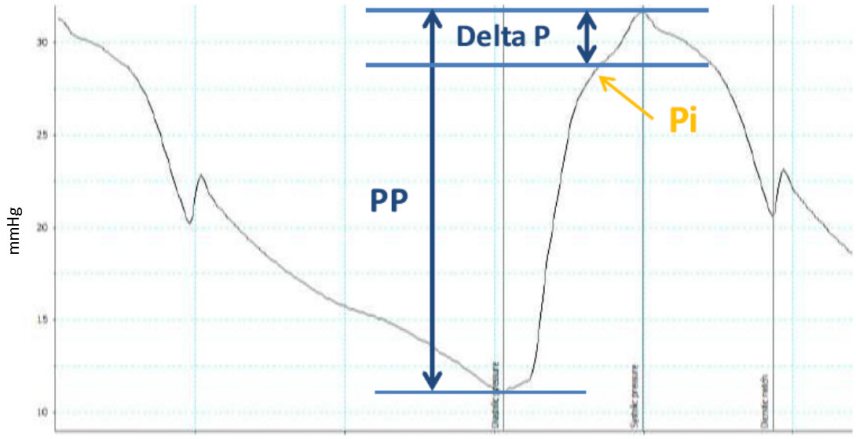


Fig. 11: Mouse pulmonary artery (PA) pressure. Note the dicrotic notch which is characteristic of the PA pressure wave

Acute PA Pressure Measurement Cont.

Pulmonary Artery (PA) Measurement and Calculation of Augmentation Index (AI)



	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Dicrotic Notch Pressure (mmHg)	Mean Pressure (mmHg)	Pulse Pressure (mmHg)	Heart Rate (BPM)
Mean	31.65	10.43	20.31	20.04	21.22	305.26

Pulse Pressure (PP)

Pulse inflection (Pi)

Delta P – change of pressure between inflection point to systolic pressure

Augmentation Index (AI)= Delta P/PP

Pulmonary artery pressure (PAP) from a rat. PAP wave detected using a solid state pressure sensor. The image describes widely available PAP parameters that can be detected and/or tabulated. Systolic pressure (SP), diastolic pressure (DP), and dicrotic notch pressure are marked on the graph and are also tabulated. Mean PAP, Pulse pressure (PP), and heart rate (HR) are displayed in the table. Longer blue arrow shows PA pulse pressure (PP) measured as difference of PA SP and PA DP. The yellow arrow shows pulse inflection (Pi), also known as the anacrotic notch. From this mark delta P arises and ends at PA SP. The delta P/PP is known as Augmentation Index (AI). AI is a measure of wave reflection and can give important information about PA stiffness. Solid state pressure sensors have supreme capacity to capture the anacrotic notch hence provide information about pulmonary artery AI.

Mouse Acute Femoral Artery Pressure Measurement

APPLICATION BASICS

Site:	Femoral Artery
Species:	Mouse
Body Weight:	over 30 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure
Catalog #:	FTH-1211B-0018

SYSTEM

SP200, SP430, ADV500

Application

Invasive femoral artery blood pressure measurement can be used to determine peripheral mean arterial pressure (MAP) and assess blood supply to the leg. Performing intravascular pressure measurement allows precise local measurement of peak, systolic, and diastolic pressure; along with more detailed analysis of systolic or diastolic durations, developed pressure, isovolumetric times, and pulse height.

Anatomical Landmarks

The femoral artery in mice is located in the area of medial thigh in direct proximity of muscle groups of mm. pectineus and adductor. The surgical dissection is done in dorsal recumbency.

Surgical Approach

Secure animal in supine position on the heating pad. Using sharp scissors, starting immediately in the medial area of thigh make a straight incision about 2 cm long, from the knee towards the medial thigh. Make the incision straight and while lifting the skin with thumb forceps, keep the scissor tips up. Using blunt Metzenbaum scissors or medium hemostats bluntly dissect an underlying subcutaneous tissue from skin (Fig.1). Instruments are parallel to the tissues bluntly dissecting with tips closed then wide open, gently separating skin from underlying tissue circumferentially around the entire incision wound.

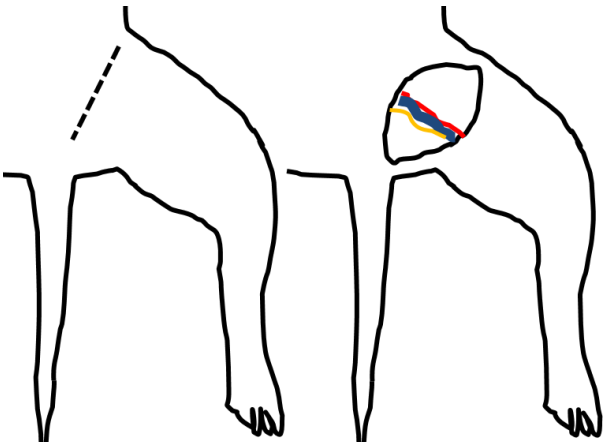


Fig. 1: Dissection of the femoral artery. A) position of the initial skin cut and B) schematic location of the fem. artery (red), fem. vein (blue) and fem. nerve (yellow). Larger vein covers the artery during the initial blunt probing and dissection.

Minor bleeding can be stopped by Q-tips or by pre-made spear shaped nitrocellulose sponges (Harvard app, QC). Keep the area moist with warm sterile saline or PBS. Gently separate via blunt dissection to expose underlying muscular layer and use retraction for visualization of the underlying femoral nerve, vein and artery. At this stage you can use fine jewelers forceps to separate medial thigh muscles and to also pierce through the membranous femoral sheath (Fig. 2).

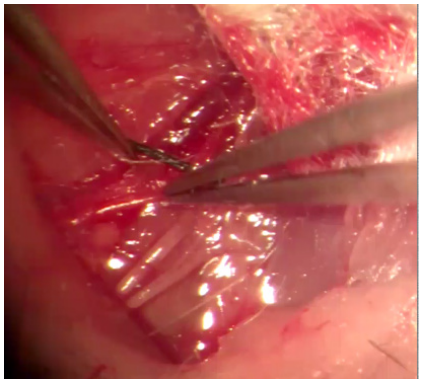


Fig. 2: Separate the thigh muscles and retract the larger femoral vein to locate the artery.

Dissect free and separate the femoral artery from the femoral vein and nerve in the area close to the inguinal ligament and follow dissection along the vessels using two fine jewelers forceps or you can use a dissecting blunt-tipped spring (e.g. McPherson-Vannas scissors) under direct microscopy visualization via at least 25x magnified field. Dissection along the femoral artery has to be done with caution as the major muscular branch called Murphy's branch has to be avoided as profuse bleeding occurs when forcefully separated. Another branch is located at about halfway down the dissection length on the femoral vein. Ensure that the section of the artery is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures.

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Mouse Acute Femoral Artery Pressure Measurement Cont.

Surgical Approach Cont.

Post-successful dissection, pass 10-0 silk suture underneath the femoral artery and ligate the distal end of the femoral artery (towards the knee) using double knots (Fig.3) and create tension on the distally placed suture end. Keep in mind a longer isolated segment will significantly improve chances for successful catheter introduction. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. Flush the area using 0.9% isotonic saline or PBS using 27-gauge Angiocath or similar (Fig.4).

Place another larger size silk 7 or 8-0 underneath the segment this time more distal order to leave enough length for the second tie securing the pressure catheter in place (Fig.5). Do not use 5-0 or larger size silk as on ligation you are able to twist the long axis of the femoral artery making it more difficult to cannulate. At the same time place microvascular clamps underneath the artery for later help with Pressure Catheter positioning. Then prepare a knot on the 7-0 silk suture (Fig.6).

Use another microvascular clamp to temporarily occlude blood flow into the segment and use a 30 gauge needle to perforate the vessel with the other underlying clamp to support the needle tip and the pressure catheter on insertion (Fig.7-9).

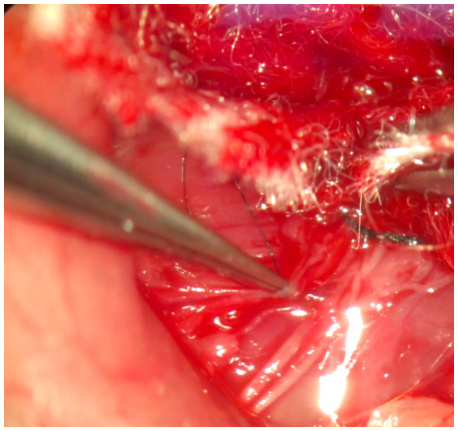


Fig. 3: Place first suture underneath the femoral artery

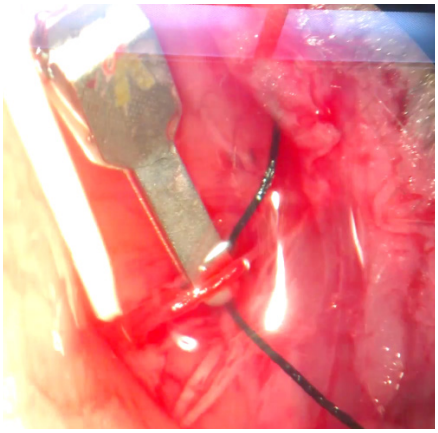


Fig. 4: Saline flush

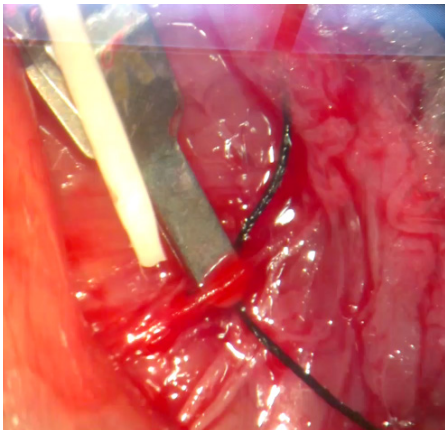


Fig. 5: Place 7-0 silk under the femoral artery and apply suction

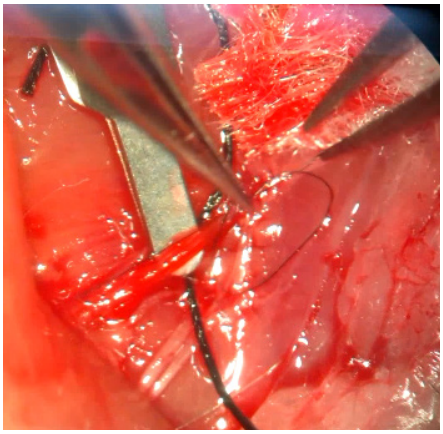


Fig. 6: Tie knots in sutures

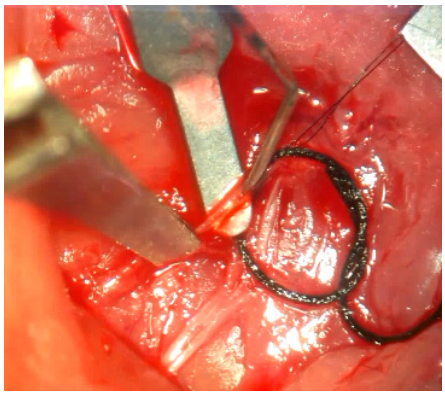


Fig. 7: Use a 30g needle to perforate vessel with vessel clamp support

Mouse Acute Femoral Artery Pressure Measurement Cont.

Surgical Approach Cont.

CATHETER INTRODUCTION (INSERTION)

Following a successful femoral arteriotomy insert the pressure catheter as close to the microvascular clamp as possible (Fig.10). Then use the clamp applicator to lift the clamp off of the segment (Fig.11) and at the same time insert with your non dominant hand the 1.2F pressure catheter into the opening, passing the pressure sensor into the area of inguinal ligament. Especially for a novice surgeon, who might take more time to successfully introduce the catheter, using the microvascular clamp might allow more time for location of the insertion into the collapsed artery, limiting blood loss from catheterization. You might position and tie off the first suture around the catheter after passing the pressure sensor in order to prevent its slipping out. At the same time, make sure there is not an excessive resistance present on introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy incision on repositioning(s). Ideally there is minimal bleeding post insertion.

Be careful not to damage the catheter with the forceps tips and hold the catheter at the same plane as the blood vessel during whole introduction see “Optimizing Catheter Life Span” on page 17.

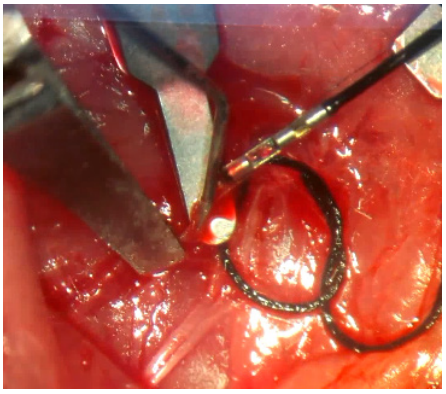


Fig. 8: Position the pressure catheter right behind the perforating needle, aligning it with long axis of the arterial segment. Perforating needle is positioned towards the inguinal ligament.

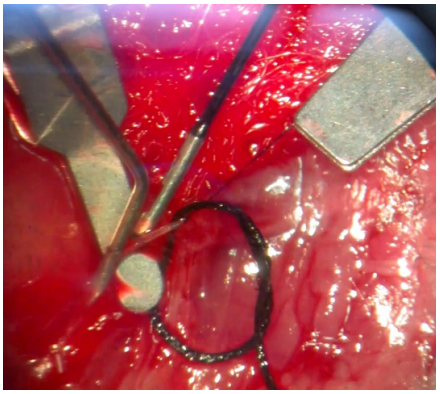


Fig. 9: Fully introduce the needle while at the same time get ready to slide in the pressure catheter immediately upon the needle withdrawal. Please note the tension created with 10-0 suture is necessary to help to straighten up the segment before catheter entry.

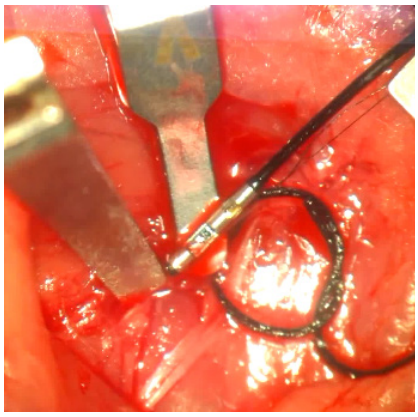


Fig. 10: Insert the catheter into the femoral artery. During catheter insertion; the catheter is positioned as close as possible to the microvascular clamp.

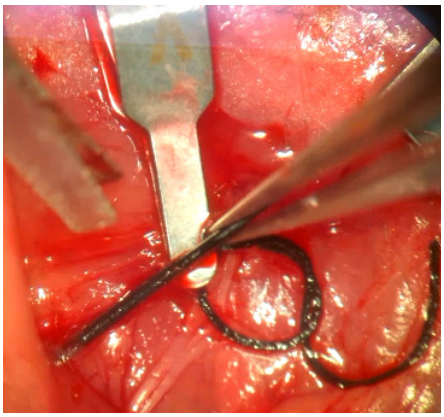


Fig. 11: Fully open the microvascular clamp and as you are removing the clamp push the catheter fully into the lumen.

Mouse Acute Femoral Artery Pressure Measurement Cont.

CATHETER POSITION ADJUSTMENT

Allow the catheter to stabilize in the artery for 5-10 min before marking the data file to your start protocol. Catheter positional adjustment needs to be made based on the acquired pressure signal. Reposition the catheter until an optimal position is found to obtain a sinusoid pressure wave (Fig.12). The software is later used to detect and mark ES (end systole), ED (end diastole), N (notch pressure), filling end or max and min DP (Fig.12).

At the end of the experiment, carefully remove the catheter by gently pulling it back through the stab wound. Immediately, insert the catheter tip into 5 ml saline pre-filled syringe. Clean the catheter as soon as possible according to proper care guidelines to considerably prolong the Catheter's life.

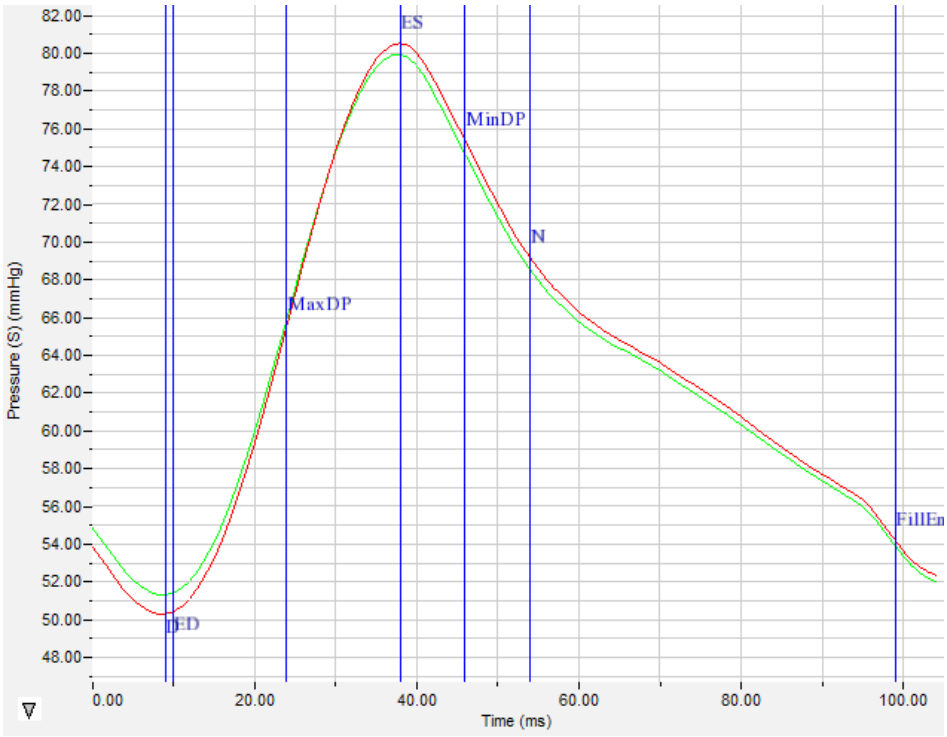
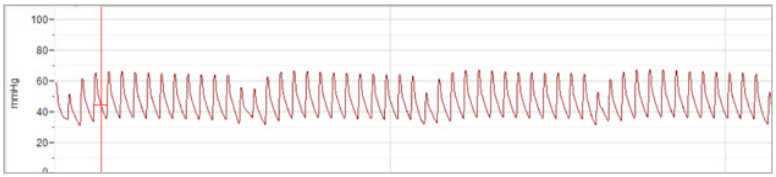


Fig. 12: Mouse Femoral Artery Pressure. Software can determine the points described on the pressure wave: ED (end diastole), ES (end systole), max and min DP (max and min pressure derivatives), N (notch pressure point), Fill End (end of filling).



Femoral pressure trace in a mouse, post induction of Ket/Xyl injectable anesthesia ventilated using 1% Isoflurane in 100% oxygen.

MOUSE FEMORAL ARTERY PRESSURE

	HR bmp	SYSTOLIC mmHg	DIASTOLIC mmHg	MAP mmHg
MEAN	605	63.85	34.35	44.18
SD	4	0.15	0.15	0.15

Rat Aortic and Left Ventricle Acute Pressure Measurement (Closed Chest Approach)

APPLICATION BASICS

Site:	Ascending Aorta and Left Ventricle - Closed Chest
Species:	Rat
Body Weight:	200 - 500 grams
Duration:	Acute

CATHETER

Size:	1.6F
Type:	Pressure
Catalog #:	FTH-1612B-0018

SYSTEM

SP200 or SP430

Application

The hemodynamic properties measured by the pressure catheter can be used to gain insight in cardiac function.

Pre-Surgical Preparations and General Anesthesia

Prepare an area for scrubbing in a separate location from where the surgical operation will take place. For cardiac surgery, it is best to find low-traffic area. Ideally, clean surfaces using disinfectants with low reaction to organic materials (e.g. Phenolics -- Lysol, TBQ).

Basic surgical supplies for rat cardiac surgery should include a sterile surgical instrument pack and sterile supplies (i.e. drapes, 4 x 4" gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask, head bonnet and sterile suture packs). In addition, a glass bead sterilizer, heating water blanket or approved electrical heating/feedback control unit should be used. Heat lamps are not ideal for body temperature maintenance and can often be a source of electrical noise/interference. Delicate rodent surgical instruments should be inspected for damage before sterilizing.

Set up surgical microscope (interpupillary distance, check light bulbs, adjust to check magnifications), organize surgical table and fine-tune surgical stool to a comfortable setting where the triangular position can be reached (both feet touching the ground with both arms comfortably resting on the surgical table). Turn on glass bead sterilizer.

Prepare 0.9% saline or a similar isotonic fluid and pre-warm the solution if it will be given pre-operatively. When a decision is made to use pre-warmed sterile isotonic fluids subcutaneously it is also suggested to use a preventive analgesia.

Before inducing anesthesia be sure to record weight, age, sex, strain, colony history and health status of each rat, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check rat's respiratory rate (65-110 breaths/min), heart rate (305-500 beats/min) and temperature (38.1-38.5°C).

Shave the animal while on the warming pad using a #40 blade attached to Oster small animal clippers (Harvard Apparatus). Remove any remaining hair from the surgical area using a depilatory cream (e.g. Nair). Apply surgical scrub alternating between disinfectant (i.e. iodophores, chlorhexidines) and alcohol. Please remember: Iodophores will inactivate a wide range of microbes, however literature describes their reduced activity in the presence of organic matter.

Use gauze squares for scrubbing. Scrubbing should always begin along the incision line and extend outwards, ensuring contaminants are not pulled towards the surgical site. Always scrub larger surface area then surgical field. Do not wet large area of skin or fur with alcohol to avoid hypothermia. Consider using drapes to maintain a sterile field and preserve body temperature.

Pre-anaesthetize rats for cardiac surgery with 3-4% Isoflurane (Forane) mixed with driving gas (Oxygen) 0.5 L/min inhaled in Plexiglas induction chamber with lid. It is important not to disturb rat during induction. Apply an ophthalmic ointment to both eyes following induction of anesthesia to prevent corneal drying.

Rat LV Acute Pressure Measurement (Closed Chest) Cont.

Pre-Surgical Preparations and General Anesthesia Cont.

Use pre-cut Styrofoam as a reclined platform with rubber band attached to the edges at the top to allow rat's neck to be situated at the top with rubber band attached to his upper incisors. Use atraumatic forceps to carefully pull out the tongue. Transorally intubate using a 16-gauge polyethylene catheter with help of fiberscope by directly illuminating ventral area of the neck. Insert catheter into the larynx past the 2 valves (vocal cords). Ventilate with tidal volume of 2.5 mL, with 85 ventilation cycles per minute. Keep the intubation catheter in alcohol between intubations for disinfection, use 50 mL syringe to clear off any residual alcohol, to avoid aspiration.

Once connected to the ventilator, inspect breathing pattern, color of membranes and capillary refill time. If feasible, use pulse oximetry. We have found that Isoflurane produces an excellent long-term controllable anesthesia for cardiac surgery. Adequate anesthesia is accompanied by loss of muscle tone and by loss of reflexes (e.g. corneal, pinnae and pedal).

Regulate post-induction anesthesia to 2% with animal placed on a warming pad (38°C) in a supine position, with the upper and lower extremities attached to the table with surgical tape. Maintain rat on 2% Isoflurane by using rodent ventilator operated in pressure-controlled mode with a maximal airway pressure of 30 cm H2O, and a positive-end expiratory pressure of 1–3 cm H2O. Prior to surgery calculate the ventilator set up. Formula is based on animal mass (Mb):

- Respiration rate (RR, min^{-1}) = 53.5 * $M_b^{-0.26}$
- Tidal volume (V_t, ml) = 6.2 * $M_b^{1.01}$

Rat Weight (g)	RR (min^{-1})	V_t
250	77	1.53
300	73	1.84

It is recommended that a "circle re-breathing circuit" with the vaporizer positioned outside of this system is used for anaesthetic delivery. Control successful ventilation by running blood gas analysis to confirm normal gas exchange.

Prior to surgery, soak the tip of the pressure catheter in 0.9% saline for ~ 20 minutes. Connect the SP200 system to the data acquisition software, ensuring all channels are calibrated. See Manual and Quick Start Guide for more details. After soaking, adjust the pressure balance to zero for atmospheric pressure.

Other methods of anesthesia may be used. Be sure to consider cardiovascular impact of anesthetic choice. Please adhere to your institutions guidelines for anesthesia and pain management. See "Rodent Anesthesia Guidelines" on page 45 for more considerations.

Surgical Approach

For right common carotid artery (RCA) access, secure animal in supine position on the heating pad. Using sharp scissors, starting immediately below the chin of the animal, make a straight incision in the direction towards the transversal pectoral muscles. Make the incision as straight as possible while lifting the skin with thumb forceps. Keep the scissor tips up. Using blunt scissors or medium hemostats, dissect any underlying glandular tissue from skin around the entire circumference of the wound. Take care to avoid major bleeding in the area. Minor bleeding can be stopped by Q-tip or gauze squares. Keep area moist with warm saline or PBS. Following this step the skin should be completely separated from underlying tissues all the way around the incision. Using medium scissors, cut as straight as possible through the fascia overlying the glandular tissue to expose underlying glands. Gently separate glands via blunt dissection to expose underlying muscular layer.

Bluntly dissect along the longitudinal right central and adjacent muscular group (sternocleidomastoid, thyrohyoid, sternohyoid, omohyoid) and remember to avoid pressure on these muscles to maintain the rat's ability to breath. Carefully separate the central muscle from parallel neck muscles and the diagonal thin muscular band (omohyoid) lying directly over the carotid vasculature. Retract skin and muscular tissues for visualization of the underlying carotid artery vasculature. Keep the

Rat LV Acute Pressure Measurement (Closed Chest) Cont.

Surgical Approach Cont.

tips of the instruments up and all tissues moist and warm. During subsequent methodical dissection and retraction of adjacent tissue, RCA can be detected next to vago-sympathetic trunk (a thin white sheath lying next to the RCA).

Continue blunt dissection to expose RCA to about 25 mm in length. Dissect alongside the RCA distally towards the head to expose RCA's bifurcations. Ensure that section of the RCA is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures. RCA must be fully separated from vascular fascia and the vagus nerve.

At this stage 5-0 sutures can be placed around RCA to be used for retraction and/or clamping and hemostasis. Use micro-forceps to place sutures around the RCA. Place the first suture as close to the sternum as possible and then place a hemostat at the end to create tension towards the tail (Fig. 1). Place another suture around the RCA and double-knot tie this suture while creating tension with a clamp and retract it towards the head (Fig. 1). At this point the RCA has been retracted proximally and distally. RCA's blood flow has been temporarily stopped. Note: Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. While creating tension on the sternal-suture, make a cut with micro-dissecting scissors in the middle of the free RCA segment. Keep in mind, a longer isolated section of the RCA will significantly improve chances for successful Catheter introduction. Next, loosely place a third 5-0 suture around the RCA and slide it towards the sternum. This suture will be tied off when the Catheter passes the first suture on the way into the aorta and heart.

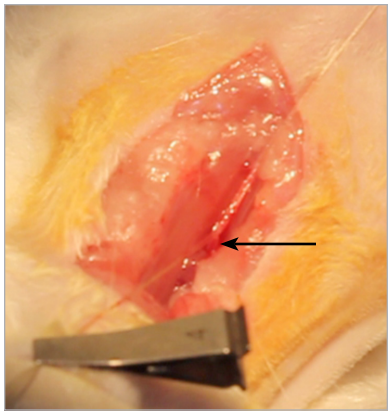


Fig. 1: Isolated RCA with sutures knotted around artery.

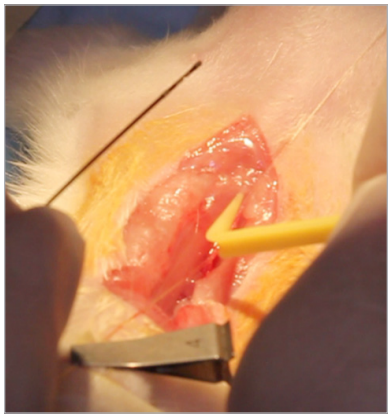


Fig. 2: Vascular inducer (yellow) is used to open the RCA in preparation for Catheter insertion.

Following a successful RCA arteriotomy use a vascular introducer to assist in opening and lifting vascular incision, while exploring the size of this opening (Fig 2). Note: Especially for a novice surgeon, who might take more time to successfully introduce the Catheter, the introducer might allow more time for the insertion in the collapsed RCA, limiting blood loss on subsequent attempted catheterizations.

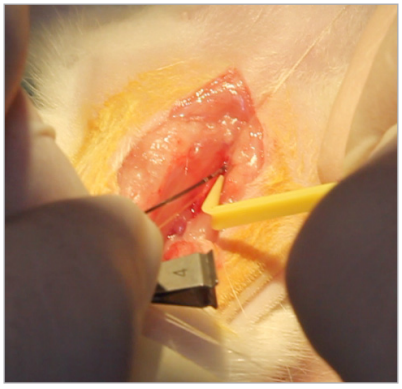


Fig. 3: Carefully remove the introducer and insert the Catheter.

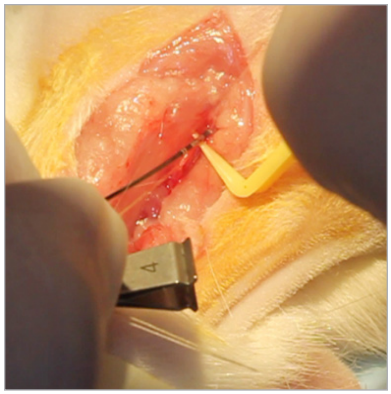


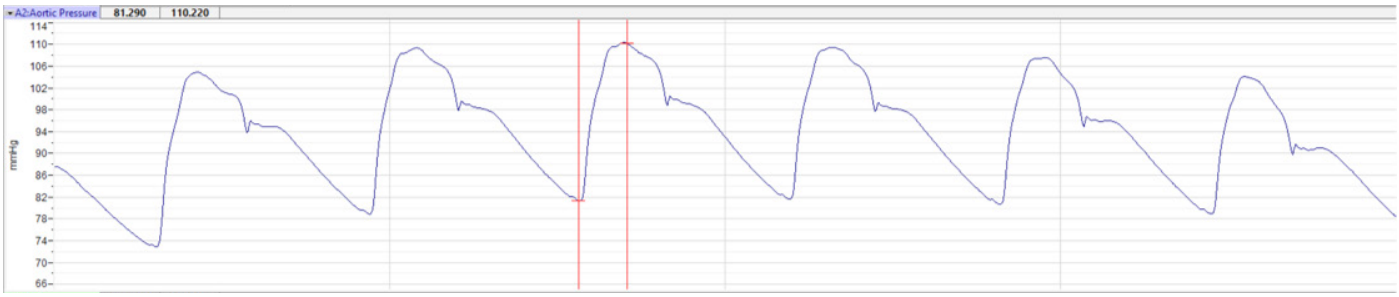
Fig. 4: Use the introducer to help Catheter insertion.

When completely contented with the RCA opening carefully proceed to insert the pressure Catheter (Fig 3). Be careful not to damage the Catheter with the forceps tips and hold the Catheter in the same plane as the blood vessel during whole introduction (Please see the instruction in "Optimizing Catheter Life Span" on page 17). Use the introducer's beveled tip to lift and level the Catheter to the same plane as the sternal RCA opening for a faster and smoother introduction into first portion of RCA (Fig 4). Make sure there is not excessive resistance present on introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy site upon repositioning. Position the Catheter and tie off the first suture around the Catheter. Ideally there

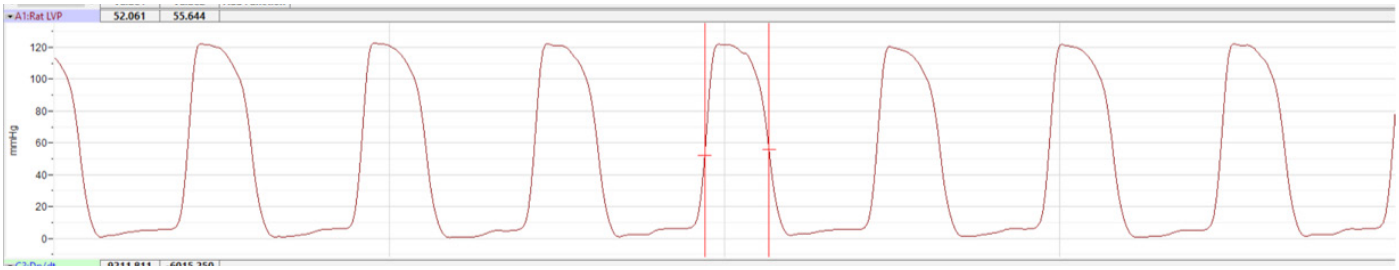
Rat LV Acute Pressure Measurement (Closed Chest) Cont.

Surgical Approach Cont.

is very low amount of bleeding. With the Catheter in the RCA, get a feel for the degree of resistance while gently rotating the Catheter in the RCA. Then tie off the third 5-0 suture around the Catheter to prevent slip out. Slide the Catheter slowly towards the heart until you see the pressure trace below for the aortic pressure and then transition to the LV pressure.



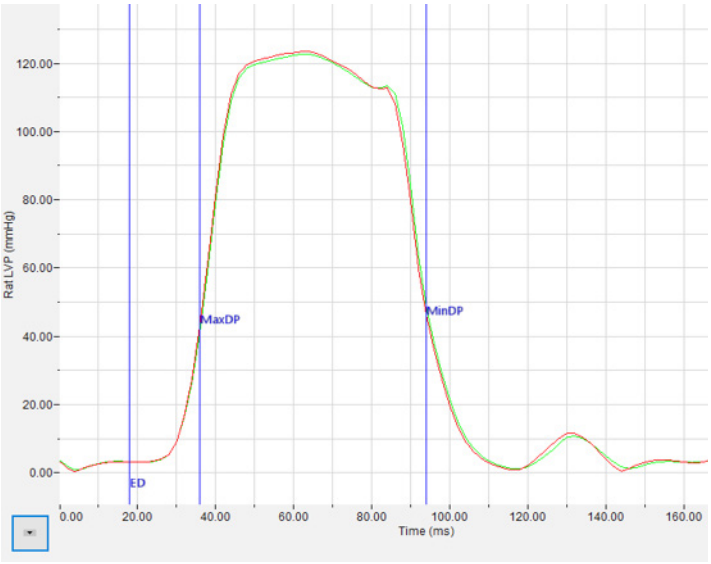
Aortic pressure waveform for rat



Left ventricular pressure waveform for rat

Single AoP waveform with marks of location of the diastolic (D); systolic (S) and end-systolic pressure (ES).

Rat LV Acute Pressure Measurement (Closed Chest) Cont.



When at rest, the majority of ventricular filling occurs prior to atrial contraction as blood passively flows from the pulmonary veins and atrial contraction contributes with lesser extent to left ventricular filling.

However at higher heart rates (410 vs. 430 bpm in our example) when there is less time for passive flows from the pulmonary veins, the atrial contraction can markedly improve ventricular filling. This atrial response is sometimes referred to as the "atrial kick."

The contraction of the left atrium might account for up to 5-30% of final cardiac output. The atrial contribution to ventricular filling varies inversely with duration of ventricular diastole and directly with atrial contractility.

Atrial kick can be associated with up to 10-12 mmHg of pressure rise. Later, as the atrial pressure starts to decline it causes a pressure gradient reversal across the mitral valve. This causes the valves to rise before its closure. At this time, the ventricular end-diastolic pressures are about 2-6 mmHg.

RAT AORTIC PRESSURE

	HR bmp	SYSTOLIC mmHg	DIASTOLIC mmHg	NOTCH mmHg	PULSE (PP) mmHg
MEAN	366.12	103.64	76.69	92.72	26.94
SD	3.81	4.18	3.18	4.63	2.98

RAT LEFT VENTRICLE PRESSURE

		HR bmp	SYSTOLIC mmHg	P_{MIN} mmHg	dP_{MAX} mmHg/sec	dP_{MIN} mmHg/sec	EDP mmHg	TAU M msec
LV	**MEAN**	410.51	121.93	1.13	5163.99	-4192.74	5.83	5.82
	SD	14.11	0.76	0.45	35.87	63.96	0.45	0.58
LV WITH ATRIAL KICK	**MEAN**	430.57	122.85	0.62	5134.85	-4706.89	3.19	6
	SD	51.52	0.99	0.52	44.8	1339.24	0.14	0

REFERENCE

Konecny, F., Zou, J., et al. Post-myocardial infarct p27 fusion protein intravenous delivery averts adverse remodelling and improves heart function and survival in rodents. Cardiovasc Res 94, 492-500 (2012)

ACKNOWLEDGMENTS

Toronto General Research Institute, McEwen Centre for Regenerative Medicine, University of Toronto, 200 Elizabeth Street, MaRS 3-908, Toronto, ON, M5G 2C4, Canada.

Keys to Successful Cardiac Output Measurements in Rodents

Common applications like the measurement of cardiac output can be challenging for the novice. Flow values that deviate from expectation may be difficult to understand and may arise from a combination of common errors. The following guidelines outline common pitfalls that affect flow readings and may present as “low” flow or “variable” flow measurement.

ASCENDING AORTA VS. PULMONARY ARTERY VS. DESCENDING AORTA

Cardiac Output (CO) is the total blood flow output of the heart. It can be measured in the Ascending Aorta or the Pulmonary Artery. In small animals (rats & mice) the pulmonary artery is much less accessible for Flowprobe placement and is rarely used unless the investigation requires assessment of blood flow distribution to the lungs.

Cardiac Output in rats and mice is directly measured with a Transonic® Flowprobe placed on the ascending aorta, just before the aortic arch. This Probe position most completely captures the total outflow of the heart on each beat, less the blood flow that is diverted into the coronary circulation (about 10%).

Estimates of CO can be measured more distal on the lower thoracic aorta or descending aorta. However, the measurement will not include flow to the head in the carotid branches or flow in the brachial arteries and forelimbs, and so would be an underestimation of cardiac output. Placement of a Flowprobe in this position is less technically challenging than on the ascending aorta, and is sometimes used to detect relative changes in CO in experiments where entering the thoracic cavity would be too invasive and not pertinent to the study design.

ANESTHETIC AGENT MAY DEPRESS CARDIAC OUTPUT

Common anesthetics used for small animal surgeries include pentobarbitol, urethane, halothane, isoflurane and ketamine/xylazine. These agents can have a significant affect on cardiac output and the effect can vary between species. These also depend on whether the measurements are to be made in acute open chest application or after recovery from implantation in the conscious animal. Pentobarbitol is known to depress cardiac output in rats, but is often used for acute experiments and CO values are lower than expected. Ketamine/xylazine is a useful anesthetic for application in rats, but has severe and lingering effects in mice. Isoflurane has fewer systemic hemodynamic side effects in the mouse and seems to preserve cardiac function. Know the effects of the anesthetic agent on the study subject.

MAINTAIN BODY TEMPERATURE

A rat or a mouse loses core body temperature quickly when a body cavity is exposed during surgery. Measurements of cardiac output will drop considerably if core temperature is not maintained. We have observed flows that are 75% lower than typical reported values in open chest preps where the temperature has deviated 9° lower than normal (example: PA flow measured acutely in a rat: 16 ml/min @ 28°C; 40 ml/min @ 32°C; 60 ml/min @ 37°C). These dramatic affects are also reported in peripheral vascular flow measurements.

Temperature should be monitored and normal body temperature maintained during the experiment. Monitoring is easily achieved with a rectal temperature sensor. Heating pads or heated surgical platforms are available or can be easily constructed to provide adequate temperature control. A heating lamp light source can also be used. In both cases, be sure not to over heat the subject. Body temperature may also be conserved by covering the exposed area with gauze.

Keys to Successful Cardiac Output Measurements Cont.

VENTILATION & APPROPRIATE SURGICAL INSTRUMENTS

Working in a small animal model requires surgical instruments that are scaled down to smaller vessels than those used for large animal surgery. See"Surgical Instruments for Acute Rodent Procedures" on page 39 for some suggestions.

Animals that undergo open chest surgery must be ventilated mechanically with a respirator that will deliver the appropriate small stoke volumes and ventilation rates that are required by small animals. There are excellent products available for this purpose from Hugo Sachs Electronik and Harvard Apparatus.

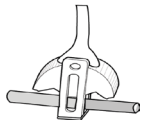
FLOWPROBE SIZE SELECTION FOR THE ASCENDING AORTA

It is important to choose the proper size Flowprobe for the animal species and strain that is used. Although Transonic® Flowprobes do fit a range of vessel sizes, the accuracy of the Flowprobe will be maximized in acute application if the Flowprobe fits the vessel more closely and the amount of gel required for acoustic signal coupling is therefore minimized. Transonic® surgical protocols recommend Flowprobe size by weight of the subject: for rats 250 – 350 grams: 2.5 mm Flowprobe; for rats < 250 grams: 2 mm Flowprobe; for larger rats 400 – 500 grams: 3 mm Flowprobe; a special 1.5 mm Probe configuration is made for a mouse ascending aorta.

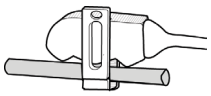
Use of a larger Probe than necessary has two potential problems. First, a larger than necessary Probe may be too large for the anatomical space within the chest cavity and the space along the vessel. Secondly, the Flowprobe ultrasonic field is not 100% uniform. PS-Series Flowprobes tend to have greater sensitivity in the center of the Probe and less sensitivity on the edges of the Probe. We specify that the vessel should fill 75 – 95% of the Probe lumen to meet our accuracy specification. A vessel that is smaller than specified for a given Probe size will exhibit variable reading depending on the position of the vessel within the Probe .

PROBE CONFIGURATION & SURGICAL APPROACH:

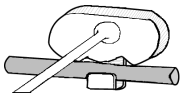
Generally, the orientation of the Flowprobe cable with respect to the Probe head ("back", "side" or "lateral") is not as critical for acute open chest protocols as it is for chronic implantation where the cable must be tunneled subcutaneously to the midscapular area. However, the cable orientation influences how the Probe is positioned on the vessel.



B- Back: perpendicular to vessel



S- Side: parallel to vessel



L- Lateral: for thoracotomy

PROBE POSITION ON THE VESSEL

The aortic arch presents a unique challenge to transit time flow measurement technology. Although Transonic® Flowprobes are largely insensitive to vessel alignment within the Probe, incorrect placement with respect to the aortic arch can have a large affect on the measurement values, causing an underestimation of flow by as much as 40%. Correct Probe position is essential to overcome this limitation: the plane of the Flowprobe ultrasonic path must be oriented perpendicular to the plane defined by the curvature of the arch for accurate flow measurement. Placing the Flowprobe so that the ultrasonic wave is in the same plane as the curvature of the aortic arch is incorrect and may cause readings that are lower than expected values. In practice, the Flowprobe will exhibit variable readings as the Probe is rotated on the ascending aorta giving the lowest reading on the curve and the highest reading when the Probe is placed correctly with respect to the arch.

Mouse Ascending Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	1.2 - 1.3 mm
Length:	2.5 - 3.0 mm

PROBE

Size:	1.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1.5PSL

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

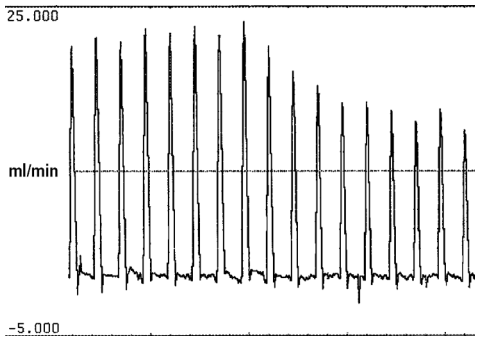


Fig. 1: Acute cardiac output measurement on ascending aorta in an anesthetized mouse.

Fig. 2: 1.5PSL Probe for mouse ascending aorta blood flow.

Fig. 3: Goldstein Lacrimal sac retractor modified (bottom) for rib retraction in a mouse.

Surgical Approach

Acute cardiac output measurements may be obtained in mice by conventional surgical techniques and ascending aorta blood flow measurements.

PREPARATION

Anesthetize the mouse with a mixture of ketamine and xylazine as mentioned in Mouse Anesthesia Guidelines (RL-67-tn). After anesthesia is attained, shave the mouse along the sternum. Place the mouse on a respirator equipped for approximately 120 breaths per minute at a very small tidal volume which is adequate to move the chest an appropriate amount for respiration. An endotracheal tube is not really necessary. Expose the trachea through a mid-line incision over the trachea. Place a 3-0 silk ligature around the trachea. Using a scalpel blade, make an incision between cartilage rings below the larynx. Pass a small polyethylene tracheal tube directly into the trachea. Connect it via silastic tubing to a rodent respirator.

STERNOTOMY

Once the animal is placed on a respirator providing positive pressure respiration, perform a median sternotomy by using scissors to cut the skin overlying the sternum. Bisect the sternum longitudinally using scissors, beginning at the manubrium and extending towards the xiphoid. Stop the incision prior to reaching the xiphoid so that the bifurcation point of the internal mammary arteries is not bisected. Try and stay as close as possible to the mid-line of the sternum to avoid cutting the internal mammary arteries.

Mouse Ascending Aorta: Acute Blood Flow Measurement Cont.

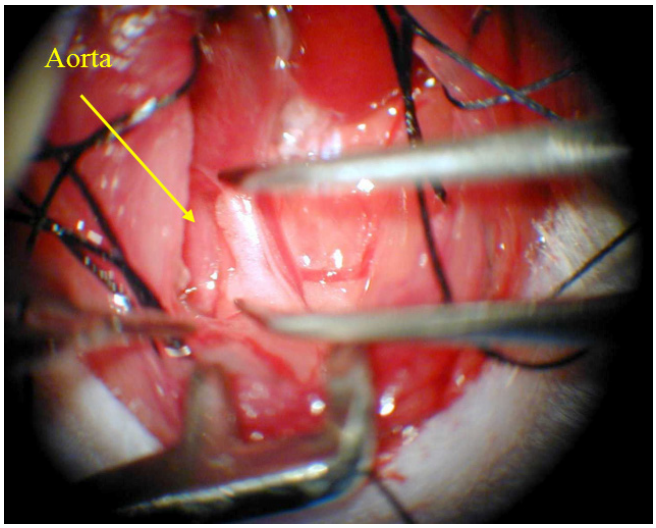


Fig. 4: Careful dissection along the ascending aorta is performed by blunt dissection using microsurgical vessel dilators to free the vessel from connective tissue.

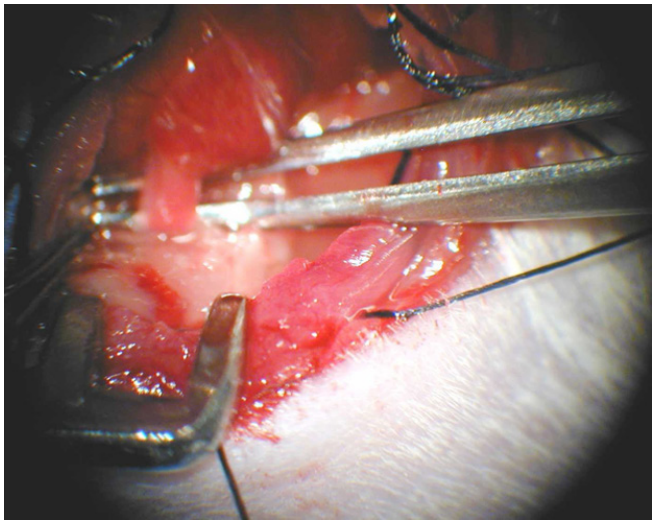


Fig. 5: The aorta has been isolated from the pulmonary artery. Gently lift under the aorta to pass surgical silk under the vessel to aid in placing the vessel in the lumen of the probe.

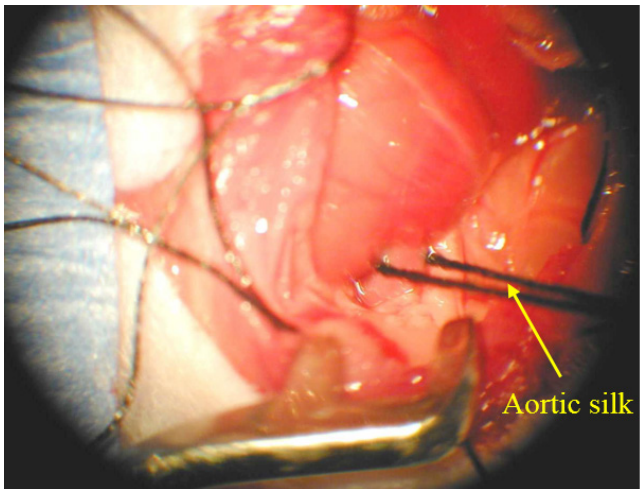


Fig. 6: Note the surgical silk around the ascending aorta at the base of the heart. This allows the aorta to be lifted into the flowprobe window.

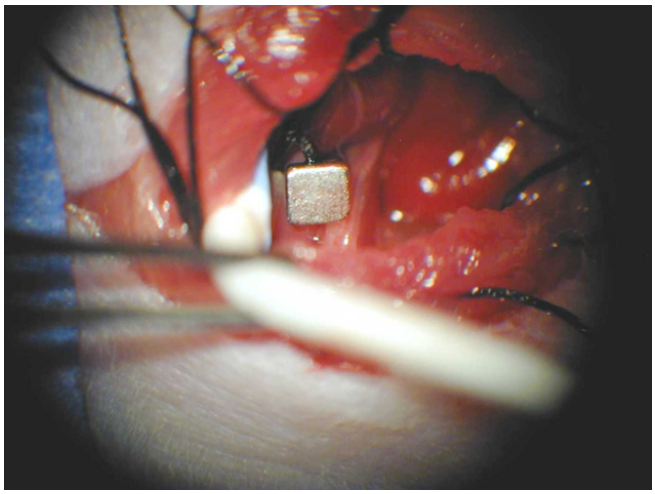


Fig. 7: The 1.5PSL flowprobe is introduced into the thoracic cavity. Then the aorta is gently lifted into the opening of the flowprobe. Confirm that the vessel is within the probe by gently rotating the probe.

Mouse Ascending Aorta: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

Once the sternum has been incised longitudinally, place a modified Goldstein lacrimal sac retractor (Fig. 3) within the incision site and retract the ribs laterally. This exposes the heart and the thymus gland of the mouse. Retract the thymus gland rostrally towards the sternum to expose the ascending aorta and pulmonary artery.

PROBE PLACEMENT

Dissect the ascending aorta free of the pulmonary artery. Position a Transonic 1.5PSL Probe perpendicular to the axis of the ascending aorta and pressed close to the origin of the aorta at the heart. This allows a long straight section of blood vessel to be illuminated with ultrasound without sampling the bend of the aortic arch. Acoustical coupling gel (Surgilube gel acoustically matches blood for the most accurate measurements and is available from Transonic®) should to be placed around the aorta, completely filling the lumen of the transit-time Flowprobe. This allows acoustical coupling of the flow Probe with the aorta itself. The Probes have been calibrated using this gel.

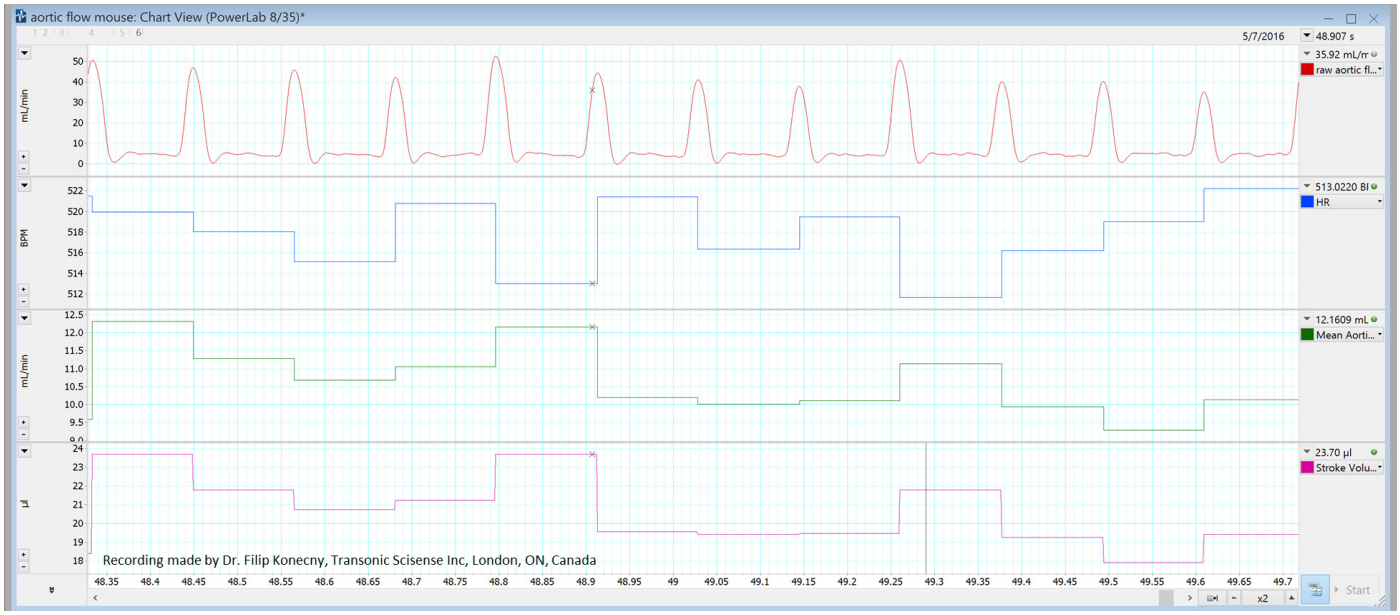
Verify zero flow and accurate positioning on the ascending aorta with a flow waveform. During diastole the flow waveform should parallel the electrical zero tracing on a recording oscillograph.

REFERENCES

Janssen, BJA et al, "Effects of anesthetics on systemic hemodynamics in mice," Am J Physiol Heart Circ Physiol 2004; 287: 1618-1624.

Gao, XM et al. "Cardiac output in mice overexpressing beta-2-adrenoceptors or with myocardial infarct," Clin Exp Pharmacol Physiol 2001; 28(5-6): 364-70.

Varghese, P et al, "β3-adrenoceptor deficiency blocks nitric oxide–dependent inhibition of myocardial contractility," J Clin Invest 2002; 106(5): 697-703.



Recording of acute mouse Aortic blood flow (Cardiac Output). Channels are:
Ch1 raw Aortic blood flow, Ch2 HR, Ch3 Mean Aortic blood flow-CO and Ch 4 is calculated channel Stroke volume (SV).

Mouse was intubated and anesthesia performed by ventilation using MiniVent type 845 (Hugo Sachs, Harvard Apparatus), Settings: tidal volume 240 µl, RR: 140 strokes/min, 100% oxygen with 1% isoflurane.

Mouse Renal Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.35 - 0.55 mm
Length:	0.25 mm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-05PSL, MA-0.5PSB

FLOWMETER

TS420 Perivascular Module

0.5PSB Nanoprobe with handle

0.5PSL Nanoprobe

Flow Ranges Observed

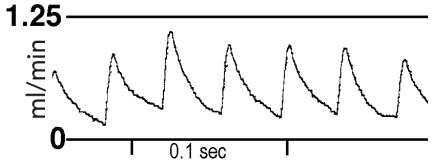


Fig. 1: Renal arterial blood flow in a 300 micron vessel in a 40 gram anesthetized mouse. Mean flow is 0.46 ml/min.

Application

The measurement of renal blood flow has an important role in research on hemodynamics, electrolyte regulation and pregnancy-induced hypertension. Flow-pressure relationships are essential in defining renal autoregulation. Other studies have focused on diuretics, cardiovascular drugs, and nephrotoxic agents. While average renal flow may also be obtained from the renal vein, the pulsatile waveform of the renal artery provides additional information and visual confirmation of a reliable renal arterial measurement.

Laparotomy surgical approaches to locating and isolating the vessel for measurement (typically used in the rat) are more challenging in the mouse. Anatomical differences from the mouse and anatomical variability among transgenic and knock out models require special consideration when choosing a surgical approach. The goals for obtaining stable data are to minimize the surgical preparation time and manipulation of the vessel and limit heat and fluid loss.

Advantages of Retroperitoneal Approach

A retroperitoneal approach to the renal artery has several advantages and is the preferred method for renal blood flow measurement. Approaching the kidney from the back allows easy visualization of the renal artery and dissection without disturbing the delicate renal vein. By laparotomy, the renal artery lies directly under the renal vein making dissection difficult.

ANATOMICAL DIFFERENCES BETWEEN MICE & RATS

Size

The renal artery of the mouse is approximately 350-550 micrometers in diameter (~60% of the diameter of the renal artery of the rat). It is ~2.5 mm long somewhat shorter that the renal artery in the rat. Less space is available for dissection than is available in the rat.

Anatomic Location

In the mouse, the renal artery differs anatomically in respect to the renal vein from the rat. In the rat, the renal artery and renal vein lie almost parallel in the same plane in the back of the animal. It is, therefore, relatively easy to dissect the renal artery away from the renal vein. In the mouse, the renal artery tends to be more dorsally positioned in respect to the renal vein. Using a conventional laparotomy, the renal artery appears to lie slightly behind the renal vein and has to be dissected free from the renal vein. This poses a challenge in that the renal vein is very thin.

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Mouse Renal Artery: Acute Blood Flow Measurement Cont.

Advantages of Retroperitoneal Approach Cont.

Retroperitoneally, there is no interference with the abdominal organs. By contrast, in laparotomy the intestines and abdominal contents are exposed and must be deflected to the side to allow access to the renal artery and vein. This lengthens the procedure and exposes the mouse's abdominal cavity for additional heat loss.

There is considerable variability in renal vascular branching among mice. In some mice, exploration of the left kidney reveal insufficient vessel length to fit the Flowprobe before the vessel branches. Because a retroperitoneal approach is quicker, it is possible to move on in the same mouse to explore the right renal artery.

Protocol: Retroperitoneal Approach, Left Renal Artery

- Anesthetize mouse and position animal in right lateral recumbency.
- Make initial skin incision 1 cm lateral to midline of back.
- Cut through skeletal muscle layer to expose the hilus of the kidney.
- Gently retract kidney to the left to expose the area between the kidney and the aorta to reveal renal artery.
- A 2 mm length of vessel without visible branching is required for Flowprobe placement.
- If the vessel is too short or bifurcates, the incision may be closed and the animal turned on its left side for exploration of the right kidney.
- Use blunt dissection along the renal artery to isolate the vessel and clear off fat for proper acoustic coupling of Probe.
- Position Probe so that the renal artery is in the lumen of the Probe.
- Use a syringe with a flexible catheter tip to deposit SurgiLube gel in air spaces of Probe and verify good transmission of the ultrasound signal by checking the Flowmeter "Test" mode.
- Stabilize Probe position with a micromanipulator for continuous measurement.

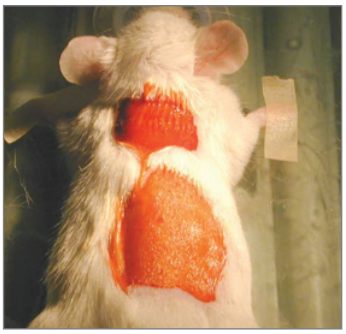

Fig. 1: Shaved, prone mouse on a warming pad prepared for surgery.

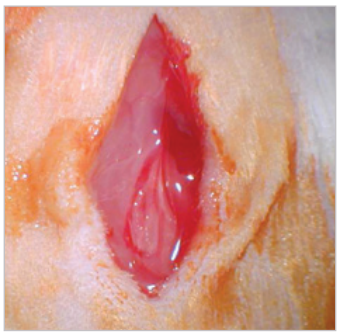

Fig. 2: Make the incision 1 cm lateral to back midline. Cut through the skeletal muscle to the hilus of the kidney.

CAUTION: CAREFUL DISSECTION REQUIRED

In general, dissections or manipulation of vessels in mice should be approached very carefully. The renal vein and renal artery may be dissected away from each other by grabbing carefully the adventitia of the renal artery and, using very fine Dumont vessel dilators (D-5az), carefully go around the renal artery and dissect it free from the renal vein. Renal artery dissections are best performed by applying slight pressure against the renal artery and allowing the D5az forceps to spread and dissect the adventitia away from the artery itself. Do not apply any kind of dissecting force against the renal vein. Instead, apply pressure toward the artery and let the instruments themselves perform the dissection by separating the adventitia from the artery. This will result in fewer misadventures with the renal vein.

ACKNOWLEDGEMENTS

Thomas L. Smith, Ph.D. & Michael F. Callahan Ph.D., Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

John Lorenz, Murine Core Physiology Facility, University of Cincinnati, Cincinnati, Ohio

Mouse Renal Artery: Acute Blood Flow Measurement Cont.

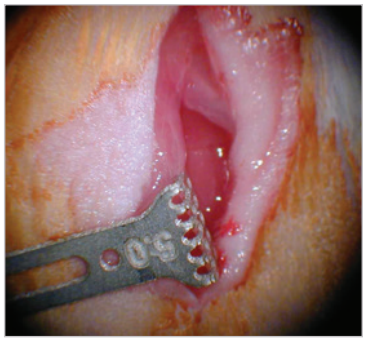

Fig. 3: Place a miniature retractor at the base of the incision to reveal the kidney beneath the muscle layer.

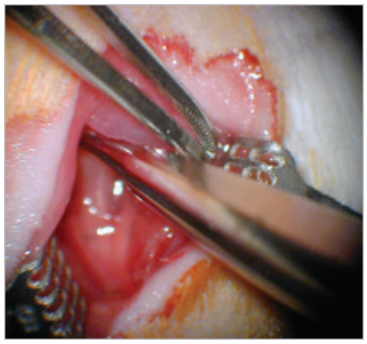

Fig. 4: Extend incision to see the renal artery. Place a second retractor and pack the kidney with gauze to keep it out of the surgical field.

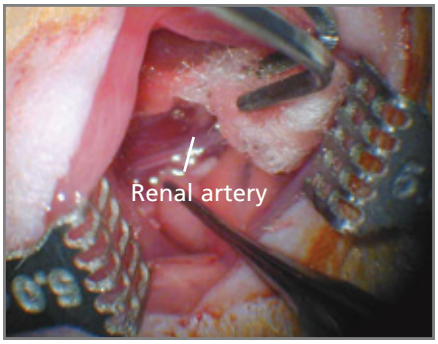

Fig. 5: Here the renal artery lies on top of the delicate renal vein. With a ventral laparotomy, the renal artery lies under-neath the vein making for a much more difficult dissection.

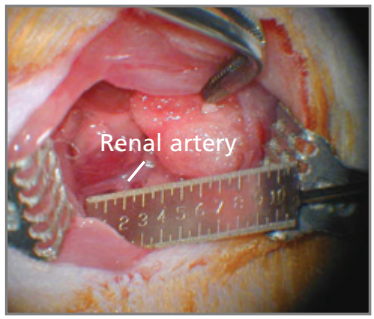

Fig. 6: This renal artery length is approximately 1.75 mm between branches, just adequate for the 0.5PSB Flowprobe.

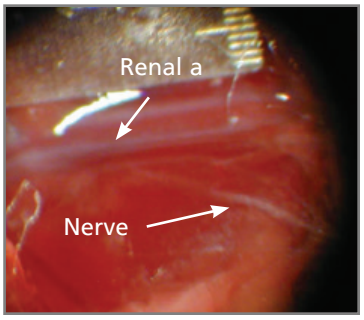

Fig. 7: Under increased magnification, the diameter of the renal artery is 0.35 mm. Note the nerve below the artery and take care to avoid damage to the nerve during dissection.

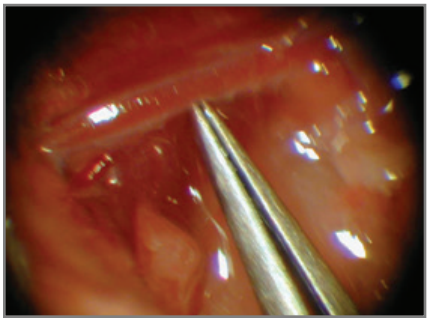

Fig. 8: Separate the renal artery from the renal vein by carefully grabbing the adventitia of the artery using very fine Dumont vessel dilators (D-5aZ) or by carefully passing microblunted 45° Dumont forceps under the vessel as shown.

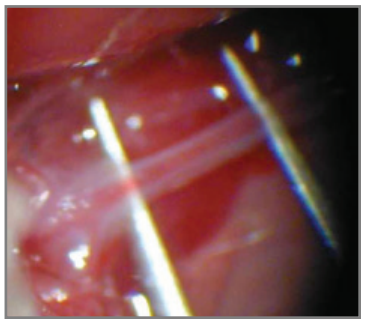

Fig. 9: Apply slight pressure against the renal artery to allow the 45° Dumont forceps to spread and dissect the adventitia away from the artery itself. Do not apply any kind of dissecting force against the renal vein.

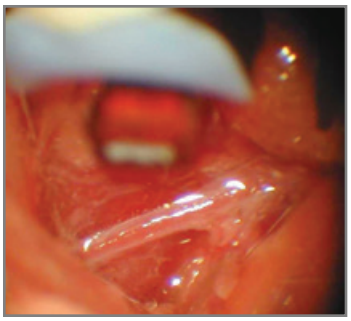

Fig. 10: Carefully go around the renal artery until a long enough segment is freed from the underlying vein to accommodate the Flowprobe reflector.

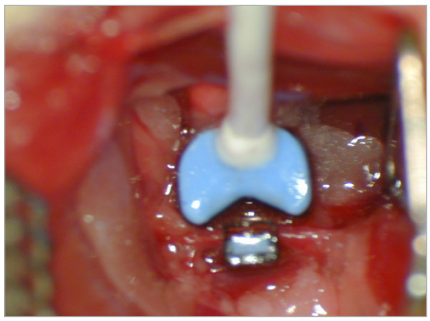

Fig. 11: Place the Flowprobe around the vessel

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Mouse Superior Mesenteric Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Mesenteric artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.60 mm
Length:	10 mm

PROBE

Size:	0.7 mm
Reflector:	JN
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

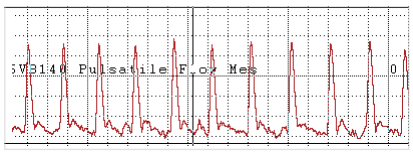
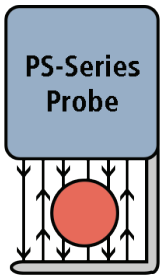


Fig. 1: Superior mesenteric arterial flow in the mouse.

Reported Flows: 0.9-3.0 ml/min in sham operated C57BL6/N male mice, body weight 25-33 g



(side view)

Fig. 2: Nanoprobe PS-Series: The full ultrasonic window has the same flow sensitivity, so that the vessel can be positioned anywhere within the Probe lumen.

Surgical Protocol

1. Administer anesthesia, clip from xiphoid process to lower abdomen.
2. Make a midline incision into abdomen (either one incision or first through skin then muscle).
3. Reflect small and large intestines laterally to the right.
   a. Major landmarks at this point include:
      - Vena cava and abdominal aorta on midline
      - Left kidney in more ventral position
      - Right kidney located more rostrally is usually obscured by intestine
      - Liver midline and superior usually partially obscures the celiac ganglia
   b. The coeliac ganglia is the large white body located in midline and slightly on the right in the area between the two kidneys. Its posterior border is perpendicular to the axis of the aorta. The ganglia contains a number of lymphatic vessels which are usually easy to visualize.
   c. The superior mesenteric artery is located within the ganglia or at its posterior border, running almost perpendicular to the aorta. Occasionally (especially in rats), it is located superiorally (under) the posterior border of the ganglia.
4. Carefully dissect the artery from the ganglia and lymphatic vessels. You should be able to clear at least a 10 mm section of the artery from the portion within the ganglia before encountering branches. Also, you can clear additional space back to the aorta. Occasionally there is a small branch near where the artery exits from the aorta.

Mouse Superior Mesenteric Artery: Acute Blood Flow Measurement Cont.

Surgical Protocol cont.

5. Place the vessel gently within the Probe lumen and attach the handle of the Flowprobe to a micromanipulator to stabilize the Probe on the vessel. Fill the space between the vessel and Probe with SurgiLube gel to aid in transmission of the ultrasound signal. Check that the "Signal Quality" indicator on the Flowmeter shows 4 – 5 lit bars; 3 or less bars may indicate an air bubble.
6. Measure flow.

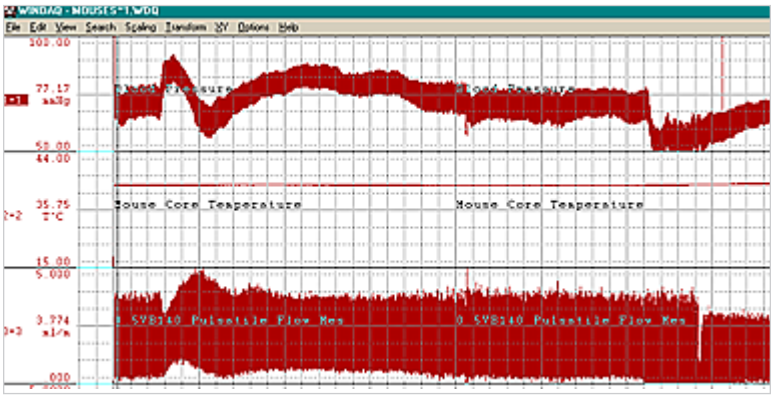


Fig. 3: Mouse Arterial Pressure (top) and Mesenteric Blood Flow measured with a 0.5PSB Nanoprobe showing dose response data.

Blood Flow Mapping in the Mouse with Transonic® Precision Nanoprobes

Ascending Aorta	1.5PSL
Lower Thoracic Aorta	1PR
Pulmonary Artery	1PR
Carotid Artery	0.5PS, 0.5VB
Femoral Artery	0.5PS
Mesenteric Artery	0.7PS
Renal Artery	0.5PS
Portal Vein	1PR

REFERENCE

Albuszies G, et al, " Effect of increased cardiac output on hepatic and intestinal mcrocirculatory blood flow, oxygenation, and metabolism in hperdynamic murine septic shock," Crit Care Med. 33(10): 2332-2338, 2005.

Mouse Femoral Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Mouse
Body Weight:	20-50 grams
Duration:	Acute
Vessel Diameter:	0.20 - 0.70 mm

PROBE

Size:	0.5 mm
Reflector:	JN
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER TS420 Perivascular Module

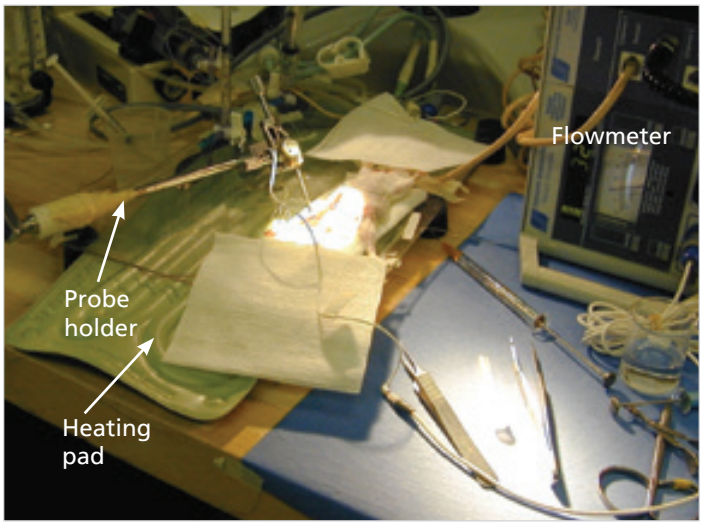
CONSIDERATIONS

Experimental Setup: Measurement Site; Vessel diameter; Maintenance of body temperature.

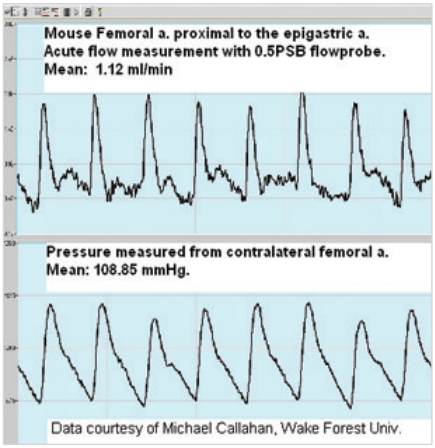
EXPERIMENTAL SETUP

Place mouse on a heating pad with lamp with gauze cover to maintain mouse core temperature. Position Probe holder with magnetic base, adjustable arm, and micro-positioning adjustment adjacent to mouse preparation. Position the Transonic® 400-Series Flowmeter Console nearby for connecting the Flowprobe.

Administer anesthesia per protocol.



Flow Ranges Observed



Simultaneous Femoral Artery Blood Flow & Pressure Recording

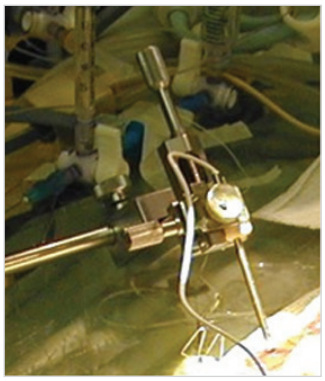


Fig. 1: Experimental Setup: Probe holder with magnetic base, adjustable arm, and micro-positioning adjustment. NanoProbe handle is clamped in a micromanipulator with two-axis adjustment to fine tune and stabilize position of Probe (see close up above). 400-Series Flowmeter Console and heating pad & lamp used with gauze cover to maintain mouse core temperature. Temperature is monitored with an anal Probe.

Mouse Femoral Artery: Acute Blood Flow Measurement Cont.

Surgical Implantation of Flowprobe

PREPARE SITE FOR FLOWPROBE APPLICATION

Prepare the medial thigh for Flowprobe application with a 1.5 cm incision into the medial aspect of the thigh near the body wall. Take care to avoid the epigastric artery which exits the femoral about 7-8 mm from the body wall and travels rostrally in a fat pad attached to the skin.

ISOLATE FEMORAL ARTERY FROM VEIN & NERVE

Use a pair of 45° microblunted Dumont forceps parallel to the vessel to open the sheath covering the femoral artery, vein and nerve. Typically, one small branch of the femoral is located between the abdominal wall and the epigastric a. Removal of the fascia reveals a 3-4 mm section of the femoral a. between the small branch and the epigastric where the Flowprobe can be placed (Figs. 4 & 5 on last page).

Grasp the fat/fascia located rostral to the vessels and pull rostrally. Isolate the nerve from the vessels with a 45° Dumont without touching or damaging the nerve. Pulling on the fascia caudal to the vein will allow it to be separated from the artery. A small branch of the femoral can usually be seen exiting caudally on the femoral artery immediately proximal to the epigastric artery.

POSITION FLOWPROBE & MEASURE FLOW (FIG. 2)

Using the micromanipulator, gently place the 0.5PSB Flowprobe in position over the vessel. Gently lift the femoral a. and place it into the lumen of the Probe. Use a syringe fitted with an angiocatheter, deposit acoustic gel in and around the vessel, being careful not to displace the vessel or damage the Probe reflector. With the micromanipulator holding the Flowprobe steady on the artery, measure flow.

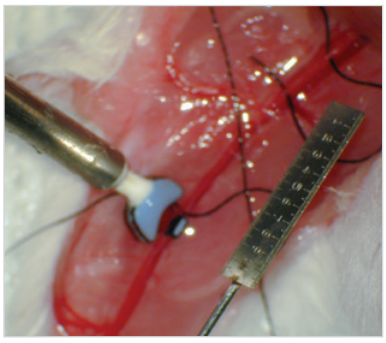
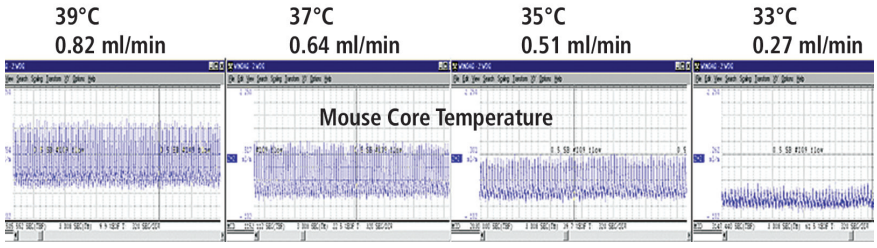


Fig. 2: A 0.5PSB Flowprobe on the superficial femoral artery, distal to the epigastric artery, must be stabilized in a micromanipulator to maintain stable Probe position during flow measurement because the vessel fills <60% of the Probe lumen. Flows at this location are ~0.6 ml/min. Recording of a femoral arterial waveform can be diagnostic of protocol difficulties. The arterial flow pulse should be apparent; an occlusion of the vessel should indicate zero flow baseline and may be nulled, as necessary to achieve true flow values.

FLOW VALUES

Blood flow in peripheral vessels can be greatly affected by core body temperature and heat loss, anesthesia, vessel spasm and Probe instability. To achieve meaningful measurements, all elements must be controlled.



Effect of core temperature on femoral blood flow in a 22 gram CD-1 mouse. Temperature has a profound effect on femoral blood flow as demonstrated by the flow traces of progressively lower core temperatures.
(Courtesy MF Callahan)

Measurement of Femoral Arterial Blood Flow in the Mouse Protocol© Courtesy of Michael F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC. ©Wake Forest University School of Medicine, Department of Orthopaedic Surgery; used with permission.

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Mouse Femoral Artery: Acute Blood Flow Measurement Cont.

Anatomical Landmarks

The segment of the femoral artery proximal to the epigastric a. is typically isolated for acute or chronic flow measurements. The vessel at this point measures ~250-300 µm in 25 g CD-1 mice. Small branches from the femoral are typically found at both locations where the 7-0 silk passes under the femoral artery.

Isolation of the artery from the vein in this position is very challenging. There is less fascia and connective tissue around the sheath of the artery and vein to manipulate the vessel free. The nerve runs immediately adjacent and is very easy to tear. Once isolated, avoid pulling up on the artery which would cause vascular spasm or putting pressure on the vein to cause occlusion.

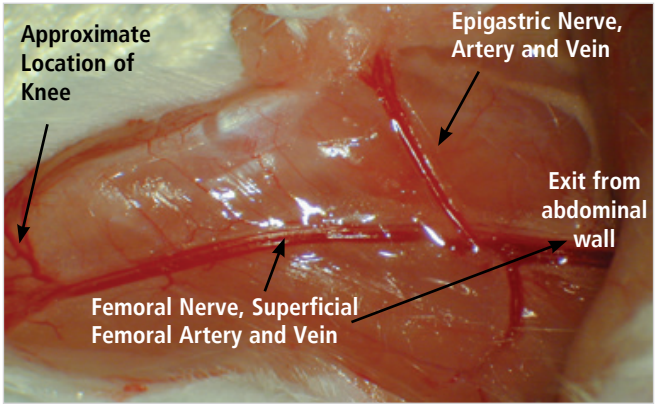


Fig. 3: Figure shows femoral nerve, superficial femoral artery and femoral vein exiting from abdominal wall and running down the leg toward the knee. Note position of epigastric nerve, artery and vein.

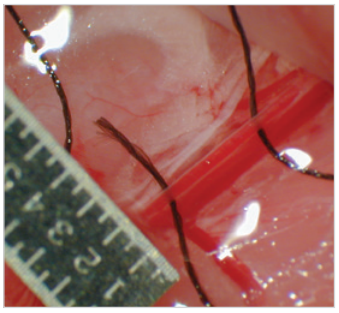


Fig. 4: The femoral artery proximal to the epigastric artery, isolated from the femoral vein, measures ~400 µm diameter with a micrometer. Flow measurements can be made at this location fairly easily.

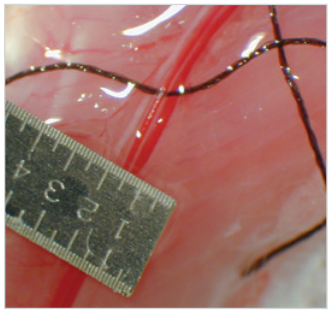
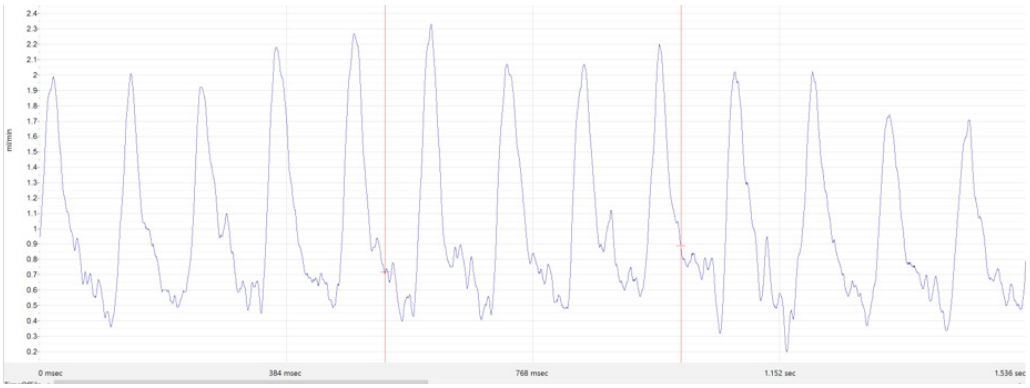


Fig. 5: The superficial femoral artery, distal to the epigastric artery, measures ~200 µm diameter and is very challenging to isolate without damaging the nerve or the vein.



Mouse femoral artery blood flow in 25 g male mouse with HR 495 BPM. Mean flow 1.1ml/min

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Fitzgerald SM, Bashari H, Cox JA, Parkington HC, Evans RG, "Contributions of endothelium-derived relaxing factors to control of hindlimb blood flow in the mouse in vivo." Am J Physiol Heart Circ Physiol. 2007; 293(2): H1072-82.

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Rat & Mouse Carotid Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Carotid artery
Species:	Mouse
Body Weight:	20-50 grams
Duration:	Acute
Vessel Diameter:	0.55 - 0.60 mm
Length:	2.5 - 3.0 mm

PROBE

Size:	0.5 mm; 0.7 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	
Smaller mice:	MA-0.5PSB
Larger mice:	MA-0.7PSB MA-0.5VB

FLOWMETER

TS420 Perivascular Module

APPLICATION BASICS

Site:	Common carotid artery
Species:	Rat
Body Weight:	280 grams
Duration:	Acute
Vessel Diameter:	0.7 - 1.2 mm

PROBE

Size:	0.7 mm; 1 mm
Reflector:	JN / JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	
Smaller rats:	MA-0.7PSB MA-0.7VB
Larger rats:	MA-1PRB w/handle

FLOWMETER

TS420 Perivascular Module

Application

The common carotid artery is an easily accessible vessel for measurements for relative cardiac output and cerebral blood flow. One advantage of the common carotid artery over the abdominal descending aorta is that the surgical approach for the common carotid artery does not require opening a major body cavity resulting in better thermoregulation and less physiological shock.

Transonic® Flowprobes are routinely used in rats and mice in standard study protocols for thrombosis formation and lysis because of their measurement precision. The recent explosion of genetic modeling techniques in mice for *in vivo* studies and the advanced technologies for bench marking results have become critical to understanding the pathophysiology of thrombosis formation and its treatment.

There are a handful of accepted methods to model arterial thrombosis in genetically altered mice. Two have been used routinely on the mouse carotid artery. The Ferric Chloride Model applies a 1 x 2 mm patch of ferric chloride-saturated paper directly on the adventitia of the isolated carotid artery. The Photochemical Model of Arterial Thrombosis uses circulating Rose Bengal that is activated by green laser illumination (545 nm wavelength) of the isolated carotid artery to cause oxygen-radical injury to the endothelium. For results that can be interpreted across studies, these methods rely on accurate "time to occlusion" blood flow measurement using Transonic® Flowmeters.

Transonic® Nanoprobes are the method of choice for carotid artery occlusive thrombosis studies which require precision measurement of zero blood flow to discern "time to occlusion". In the literature they are often mistaken for "Doppler Probes." They are not Doppler velocity Probes. Transonic® Nanoprobes are miniaturized ultrasonic transit-time Flowprobes that loosely cradle the target vessel in the mouse and directly measure the volume flow of blood in the vessel in ml/min. They are sized for mouse-size vessels and mouse-sized flow rates. Mouse carotid flow rates average approximately 0.24 to 0.7 ml/min in sham operated pre-occluded experimental animals depending on anesthetic and protocol that includes or does not include mechanical ventilation.

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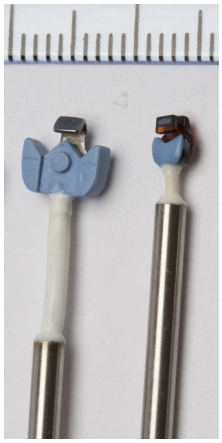
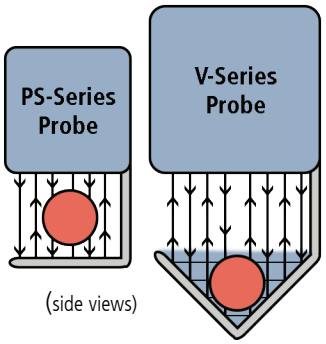
Rat & Mouse Carotid Artery: Acute Blood Flow Measurement Cont.

Introduction

The carotid artery is a long vessel that is free of branches and very easy to locate and isolate. In a mouse, the vessel is 0.5 to 0.6 mm diameter. Transonic® 0.5PSB and 0.7PSB Nanoprobes fit the vessel very closely, thereby minimizing the amount of acoustic gel required to achieve and maintain good signal. These small bodied Probes allow best visualization of the vessel and experimental site since they occupy little space along the vessel. Nanoprobes for acute use are fitted with handles, useful in stabilizing the position of the Probes for precision experiments such as measuring the time to occlusion in thrombosis studies.





PS-Series Nanoprobe: The full height of the ultrasonic window has the same flow sensitivity, so that the vessel can be positioned anywhere within the Probe lumen.

V-Series: Only within the triangle (shaded) portion of the V will the Probe reach its full flow sensitivity

Fig. 1: Side-by-side comparison of a V-Series Flowprobe (on left) and NanoProbe (on right).

In a rat, the vessel is minimally 0.7 mm diameter to 1.2 mm diameter, depending on the age of the animal and the treatment protocol (eg. ligation of the contralateral carotid artery) A 0.7PSB Nanoprobe will fit the vessel very closely and may be used in smaller animals, thereby limiting the amount of acoustic jelly that is required to achieve good signal. However, the Nanoprobe will be constrictive on many animals. For vessels larger than 0.7 mm diameter, use a 1 mm 1PRB Flowprobe fitted with a handle.

Many studies also reference high sensitivity 0.5VB mouse and 0.7VB rat Flowprobes for thrombosis applications. These may be used in this vessel location since the carotid is a long vessel and affords the space for the larger bodied Probe. If using the V-series, make certain that the vessel is fully within the sensitive "V" area of the Probe reflector and fill the remaining space with acoustic gel (Fig. 5). Only the "V" area of the Probe is sensitive to total flow. Flow outside this "V" position will be underestimated.

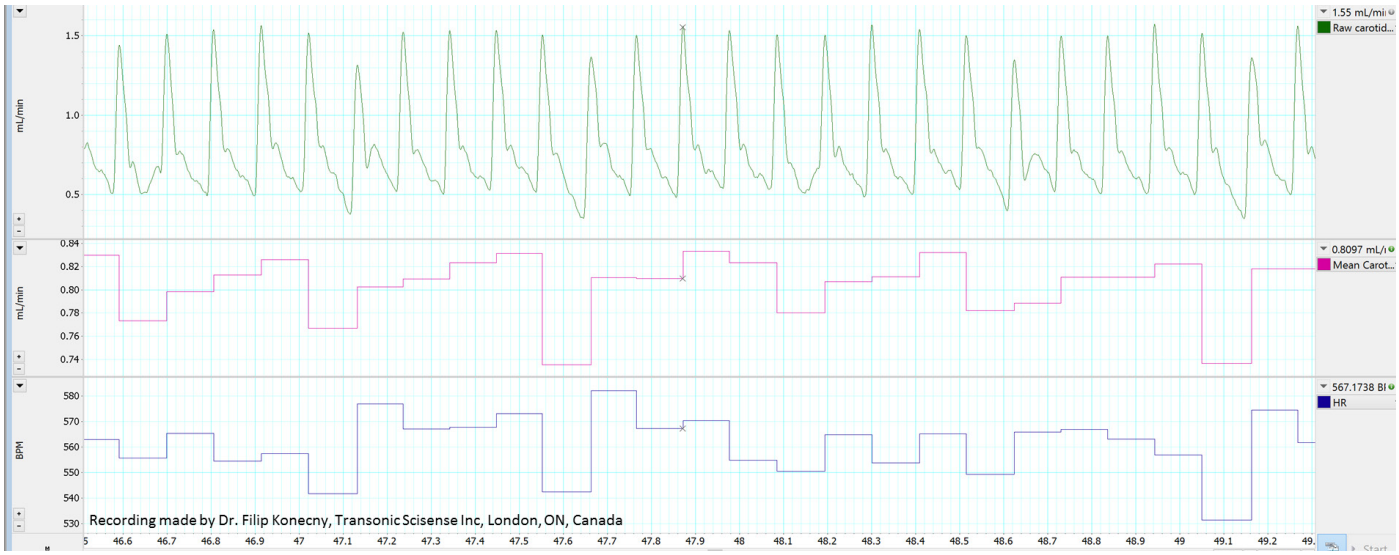


Fig. 3: Acute Right Carotid artery blood flow wave recording from a mouse. Typical carotid artery blood flow wave is can be seen with presence of breathing artifact during its recording on multiple occasions. Channels are: Ch1 raw right carotid artery blood flow, Ch2 Mean Carotid artery blood flow, Ch3 HR. Mouse was pre-anesthetized with 4% isoflurane in Plexiglas chamber and then face mask/ fitted nose cone was used for delivering volatile gas anesthesia with 100% oxygen (2.5l/ min flow) and 1% of isoflurane, during blood flow measurements.

Rat & Mouse Carotid Artery: Acute Blood Flow Measurement Cont.

Surgical Approach

Mice are anesthetized with sodium pentobarbital (70-90 mg/kg intraperitoneally) and secured in a supine position under a dissecting microscope. A heating pad is used to maintain body temperature at 36-37 °C. Supplemental anesthesia is administered as needed. A midline cervical incision is made and the trachea and right common carotid are dissected free, Mice are ventilated mechanically with room air and supplemental oxygen (80 breaths per minute, stroke volume 0.5 ml) using a Harvard rodent respirator modified with a 1.0 ml cylinder and piston assembly. Carotid artery blood flow is measured with a 0.5 PSB Flowprobe.

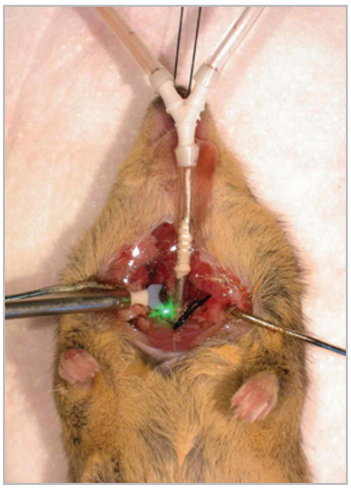


Fig. 4: Ventilated mouse undergoing photochemical thrombosis in the carotid artery.

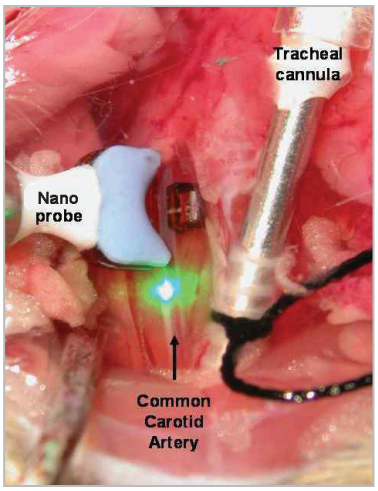


Fig. 5: Close-up of 0.5PSB Nanoprobe measuring carotid arterial blood flow in the mouse.

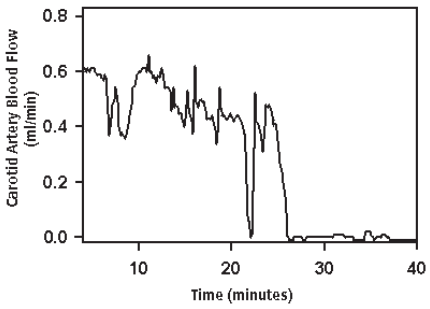


Fig. 3. Photochemical-induced injury set-up: Green laser light illumination on isolated mouse carotid artery proximal to Transonic Systems 0.5PSB Flowprobe. Sustained zero blood flow measured by the Flowprobe indicates carotid artery occlusion from thrombosis.

ACKNOWLEDGEMENT

Data and pictures courtesy of Katina Wilson and Steven Lentz, Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IO.

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Rat Ascending Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Rat
Body Weight:	230 grams
Duration:	Acute
Vessel Diameter:	2 mm

PROBE

Size:	2 or 2.5 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	100 cm
Catalog #:	MA-2PSB or MA-2.5PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

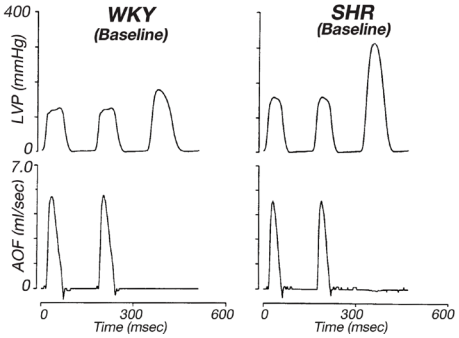

Fig. 1: A typical example of left ventricular pressure (LVP) and aortic flow (AOF) data from in-situ experiment. Base-line data are depicted for 2 steady-state contractions followed by an isovolumetric contraction obtained by occluding ascending aorta in diastole. Left and right panels correspond to data for Wistar-Kyoto (WKY) and spontaneous hypertensive rat (SHR), respectively.

Note: Instantaneous flow in a ketamine anesthetized rat peaked at over 300 ml/min. Cardiac index was 166 ml/kg/min. These values are substantially higher than those in rats anesthetized with pentobarbital.

Application

Measurement of cardiac output has many applications. One researcher studies vasoconstrictors by combining CO with pressure and flow measurements in peripheral vessels. Another studies the effect of altering isomyosin composition on left ventricular resistance. This application requires high speed (1000 Hz) data acquisition and sophisticated digital signal processing.

Surgical Protocol

Anesthetize the rat with ketamine hydrochloride (70 mg/kg IP) and mechanically ventilate. Perform a median sternotomy and open the pericardium taking care to avoid the vagus nerve. Accuracy is greatest when the Flowprobe fits the vessel very closely and a minimum of acoustic gel is needed.

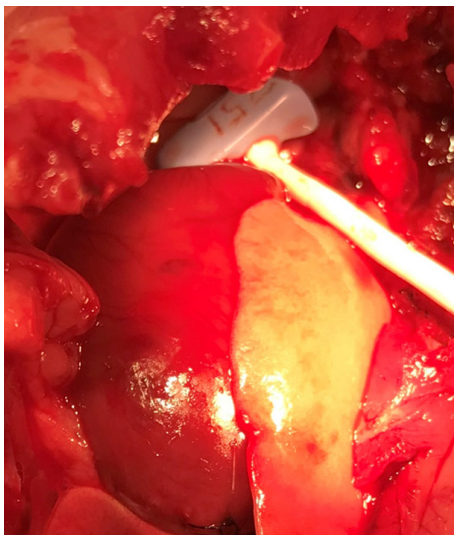

Fig. 2: 2.5PSL Probe on Aortic Arch from open chest approach

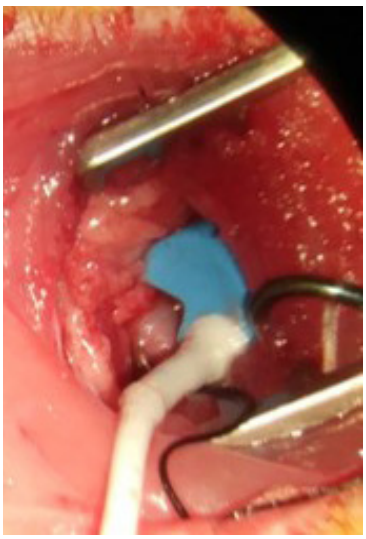

Fig. 3: Alternative closed chest approach for 2.5PSL probe on the Aortic Arch

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Rat Ascending Aorta: Acute Blood Flow Measurement Cont.

Surgical Protocol cont.

Place the bracket around the ascending aorta just above the coronary arteries. If you are using a Probe with a slide, close the slide. Position the Flowprobe as shown in Fig 4. In this location, the Flowprobe is perpendicular to the curvature of the arch. Incorrect placement is shown in Fig 5. In Fig 5, the sound beam of the Flowprobe is parallel to the arch and flow may be significantly underestimated.

We recommend Surgilube gel as a couplant because its acoustic velocity is within 30 m/sec of blood. Most ultrasonic coupling gels have an acoustical velocity different than blood and tend to lower sensitivity. To apply gel, remove the plunger of a 30 cc syringe and load the syringe with sterile lubricating gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The lubricating gel must replace all air space to be effective as an acoustical couplant. Select test mode on the Meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of gel or an air bubble.

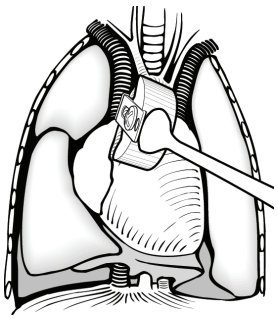

Fig. 4: Correct Flowprobe position

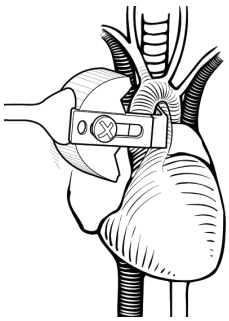

Fig. 5: Incorrect Flowprobe position

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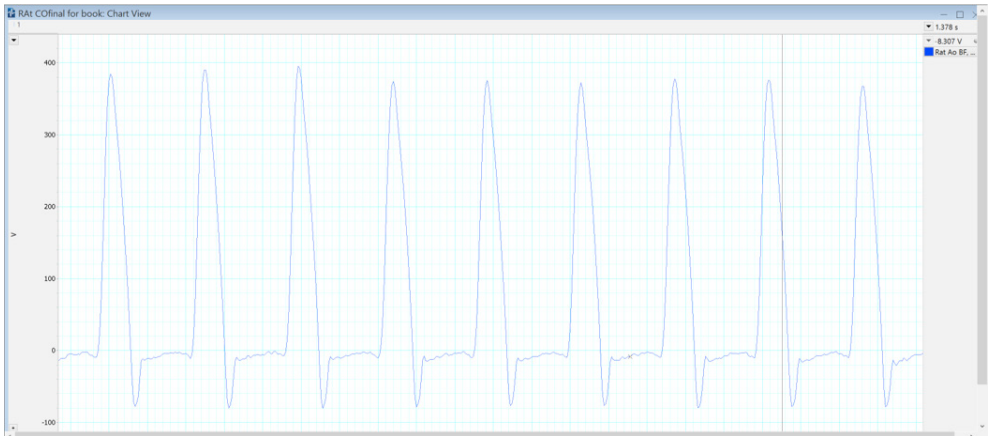

Aortic blood flow in a 347g, male rat under 1.5% isoflurane. Mean flow: 70-72 ml/min

Rat Abdominal Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Abdominal aorta
Species:	Rat
Body Weight:	270 grams
Duration:	Acute
Vessel Diameter:	1.2 mm

PROBE

Size:	1.5 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1.5PRB

FLOWMETER

TS420 Perivascular Module

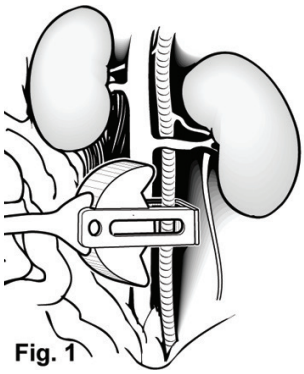

Fig. 1: Application of Flowprobe on the Abdominal Aorta.

1.5PRB

Application

The measurement of blood flow on the abdominal aorta is commonly used by researchers for protocols that require relative cardiac output, vascular resistance or absolute flow to the hind limbs. The surgical approach to the abdominal aorta is less difficult than the corresponding approach to the ascending aorta and does not require mechanical ventilation.

Surgical Approach

Anesthetize the rat with ketamine/xylamine solution (0.09 ml/ 100 gm body weight IM, thigh). The use of a heating pad or hot water bottle is recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended.

Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the rat's right to expose the abdominal aorta and the left kidney. Carefully dissect free a 1 cm segment of the aorta just caudal to the kidneys. Remove adjacent fat for proper acoustical coupling. Place the Probe around the artery and close the slide. Manually position the Probe so that the artery is centered within the window and then tape down the Probe cable to help stabilize the Probe. If there is sufficient connective tissue, the Probe may also be sutured in position.

Remove the plunger of a 30 cc syringe and load the syringe with SurgiLube gel, taking care to prevent the formation of air bubbles. Place a flexible catheter (or angiocatheter) on the tip of the syringe; the catheter may be inserted into the Probe's acoustic window adjacent to the vessel and the gel deposited as the syringe is withdrawn. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

ACKNOWLEDGEMENT

Flow trace courtesy of Dr. Wayne Schwark, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

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Validation

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Rat Common Iliac Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Common iliac artery
Species:	Rat
Body Weight:	300 grams
Duration:	Acute
Vessel Diameter:	0.7 - 0.9 mm

PROBE

Size:	1 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1PRB

FLOWMETER	TS420 Perivascular Module

Surgical Approach

Anesthetize the rat with ketamine/xylazine (0.09 ml per 100 gm body weight) in the thigh. The use of a heating pad or hot water bottle is also recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended. Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend abdominal incision through the linea alba into abdominal cavity.

Carefully locate the iliac artery which bifurcates at the terminal of the abdominal aorta. Use blunt dissection with forceps to isolate @ 0.7 mm of the artery from the vein. Place the Probe around the artery (Fig. 2). Manually position the artery so that it is lies within the lumen of the ultrasonic window of the Probe. Then tape down the Probe cable to help stabilize the Probe. Probes with a handle may be stabilized with a micromanipulator.

Apply acoutical couplant by removing the plunger of a 30 cc syringe and load the syringe with sterile Surgilube gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The gel acts as an acoustical couplant and must replace all air space. Select test mode on the meter to verify that signal amplitude is about 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

ACKNOWLEDGEMENT

TL Smith, PhD, Department of Orthopedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

Flow Ranges Observed

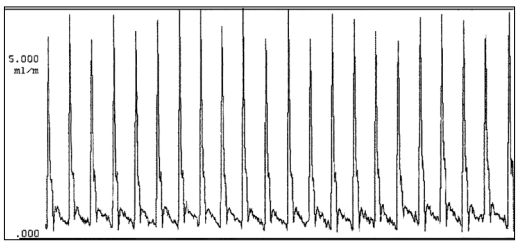

Fig. 1: Iliac flow in the rat varies greatly with the anesthetic used and the plane of anesthesia. In general, protocols with ketamine will show higher flows than those with pentobarbital. Hypothermia is also a common cause of lower than expected flow measurements.

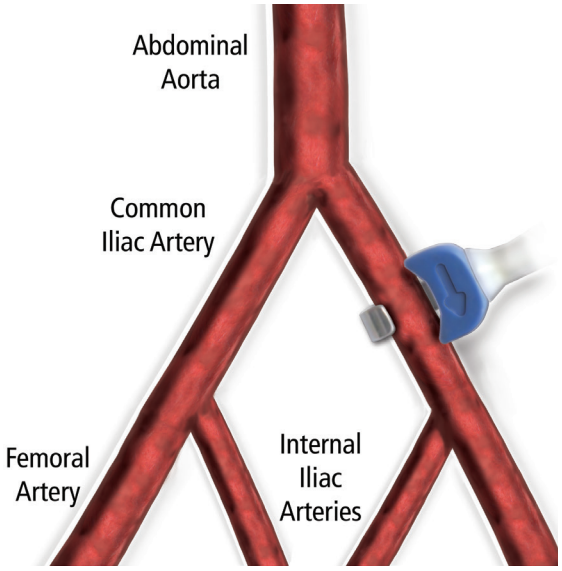

Fig. 2: Flowprobe on common iliac artery

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Rat Renal Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Rat
Body Weight:	< 300 grams
Duration:	Acute
Vessel Diameter:	0.7 - 0.8 mm

PROBE

Size:	1 mm
Reflector:	V / JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7VB, MA-1PRB

FLOWMETER

TS420 Perivascular Module

1PRB

0.7VB

Flow Ranges Observed

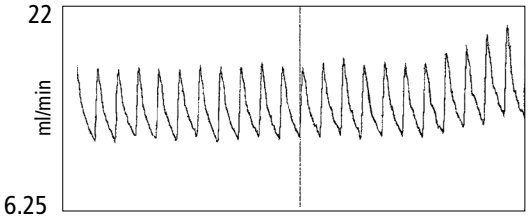

Fig. 1: Renal flow in the rat varies greatly with the anesthetic used and the plane of anesthesia. In general, protocols with ketamine will show higher flows than those with pentobarbital. Hypothermia is also a common cause of lower than expected flow measurements.

Application

The measurement of renal blood flow has an important role in research on hemodynamics, electrolyte regulation and pregnancy induced hypertension. Flow-pressure relationships are essential in defining renal autoregulation. Other studies have focused on diuretics, cardiovascular drugs, and nephrotoxic agents. While average renal flow may also be obtained from the renal vein, the pulsatile waveform of the renal artery provides additional information and visual confirmation of a measurement with a properly functioning Flowprobe.

Surgical Approach

Anesthetize the rat with Inactin anesthesia (100 mg/kg IP). If Inactin is not available, sodium pentobarbital (60 mg/kg IP) may be used instead. Note that pentobarbital anesthesia is less stable than Inactin and may significantly depress flow when compared to that of the conscious animal. The use of a heating pad or hot water bottle is recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended (See Anesthetic Guideline for more details).

Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the rat's right to expose the left kidney. To expose the right kidney, deflect the intestines to left. Identify the large renal vein; the renal artery is much smaller, cranial and deep to the vein. Carefully locate and dissect out the renal artery. Remove adjacent fat for proper acoustical coupling. Place the Probe around the artery (Fig. 2).

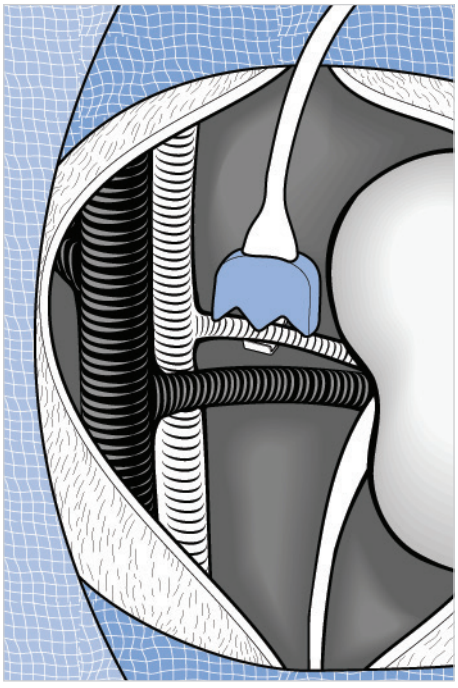

Fig. 2: Flowprobe on renal artery.

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Rat Renal Artery: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

Manually position the artery so that the vessel lies within the lumen of the Probe. A micromanipulator may be used to stabilize the Flowprobe position on the artery.

Remove the plunger of a 30 cc syringe and load the syringe with Surgilube acoustic gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The lubricating gel acts as an acoustical couplant and must replace all air space. Check the signal bar indicator on the Meter to verify that signal amplitude is about 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

ACKNOWLEDGEMENTS

Dr. Brian Murray, Department of Medicine, Nephrology Division, State University of New York at Buffalo, Buffalo, NY

Dr. Thomas L. Smith, Department of Orthopedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

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Rat Hepatic Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Hepatic artery
Species:	Rat
Body Weight:	430 grams
Duration:	Acute
Vessel Diameter:	0.25 - 0.4 mm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER	TS420 Perivascular Module

Application

In other species, measurement of hepatic arterial blood flow is often combined with portal vein flow for studies on nutrition, septicemia and toxicology. Since the net flux of a metabolite is the product of its arteriovenous or portovenous concentration difference and blood flow, the net hepatic uptake (or secretion) of any metabolite may be determined with three sampling catheters and two Flowprobes.

Hepatic arterial flow is also an important parameter in several models of liver transplant. In one study in pigs, hepatic arterial flow increased at the expense of portal vein flow following liver denervation. In humans, the routine intraoperative measurement of hepatic arterial flow in pediatric liver transplants has been advocated as a means of predicting early hepatic arterial thrombosis.

Surgical Approach

Anesthetize the rat with pentobarbital anesthesia (20 mg/kg IP). Note that pentobarbital anesthesia may significantly depress flow when compared to flow in the conscious animal (See Anesthetic Guidelines RL-67-tn for more information). The use of a heating pad or heat lamp is recommended as hypothermia also reduces flow. A 0.5 ml bolus of saline placed subcutaneously every half hour is also recommended.

Place the rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Retract the lobes of the liver cranially to locate the splanchnic vessels. Locate the pulsing hepatic artery where it branches from the short celiac artery.

The hepatic artery is easily dissected from adjacent tissue at this site as there is no immediately adjacent vein. However, at this site the hepatic artery does have flow components destined for the stomach and small intestine. For a more accurate assessment of hepatic flow, it may be necessary to trace and dissect the proper hepatic artery

Flow Ranges Observed

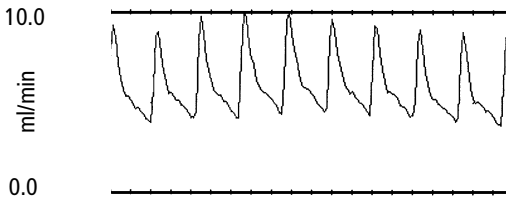

Fig. 1: Instantaneous flow in this anesthetized 330 gm rat ranged from 5 to 10 ml/min. The pulse rate was approximately 380 bpm. This measurement was made directly on the hepatic artery and includes the flow components going to the gastroduodenal and right gastric arteries.

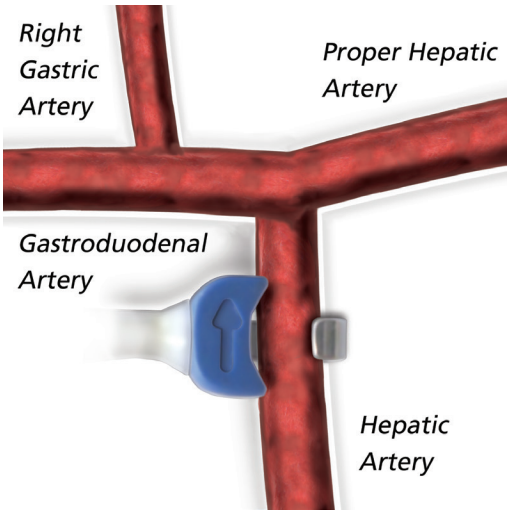

Fig. 2: Schematic of Flowprobe on a rat hepatic artery.

Rat Hepatic Artery: Acute Blood Flow Measurement Cont.

from the portal vein or ligate the right gastric and gastroduodenal branches. Clean off the fat on the vessel for proper acoustic coupling and place the Probe around the vessel making sure that the vessel is within the lumen of the Probe ultrasonic window. For extended measurements, a micromanipulator can be used to maintain proper position of the Flowprobe.

Fill the plunger of a 30 cc syringe and load the syringe with sterile Surgilube acoustic gel, taking care to prevent the formation of air bubbles. Place a flexible 20 gauge catheter on the tip of the syringe. Insert the catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The Surgilube gel acts as an acoustical couplant and must replace all air space. Check the signal bar indicator on the meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or to an air bubble.

Rat Mesenteric Branches: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Mesenteric branches: jejenal & ileal arcades
Species:	Rat
Body Weight:	400 grams
Duration:	Acute
Vessel Diameter:	270 µm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER

TS420 Perivascular Module

Application

The combination of this model and the new microcirculation transit- time Probe, allowed us to make the first measurements of true flows in the microcirculation. These were until now estimated from diameters and velocity measurements; this method lacked precision and prevented any appreciation of the instantaneous dynamic of flow. This allowed us to examine the instantaneous dynamic and to observe the cyclic variation of flow caused by the periodic contractions of the bowel.

Surgical Approach

Note: This protocol is for mesenteric branches, jujunal and ileal arcades. The superior mesenteric artery (0.7 - 0.9 mm diameter) is larger than the branches and requires 1 mm Probes for full ultrasonic illumination.

Male Sprague-Dawley rats weighing approximately 400 gm were anesthetized with 0.1 ml / 100 gm pentobarital (6%) i.p. Anesthesia was maintained by additional i.p. injections of diluted pentobarital. The right jugular vein was cannulated with polyethylene tubing for the administration of drugs.

After a small abdominal incision, a section of the ileum was pulled out and spread over the transparent stage. Fat and connective tissues surrounding the mesenteric arteries (internal artery diameters were approximately 270 µm) were carefully removed under a dissecting microscope.

The preparation was mounted under a biological binocular microscope (Leitz) connected to a color video recording system. The surface of the mesenteric artery was covered with a Saran film. Warm Krebs' solution was superfused on the artery at a rate of 2 ml/min. The microscope magnification was 145 fold. The stainless steel handle of the Probe was connected to a micromanipulator in order to allow positioning of the Probe around the vessel near the objective. The vessel was positioned in the lumen of the Probe, making sure the reflector bracket was not tugging on the vessel wall to reduce the flow. Zero flow reading was obtained by transient clamping of the arteriole under investigation.

Basal flow measurements were done at a few minute intervals, the mean flows over 90 seconds were 0.494 ± 0.276 ml/min and 0.527 ± 0.233 ml/min respectively. The reproducibility seems excellent despite the elevated standard deviation due to the physiological periodic reduction of flow provoked by intestinal peristalsis.

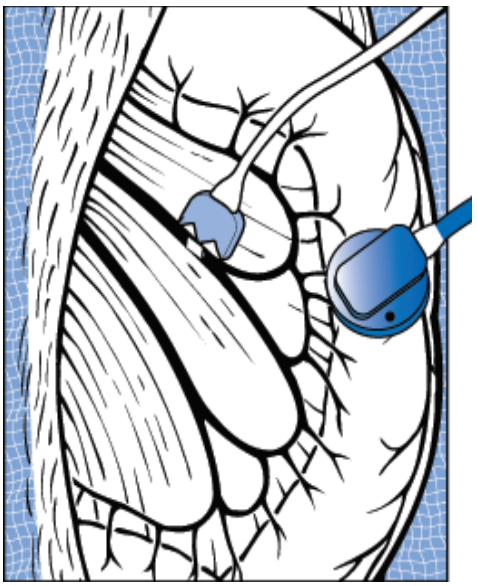

Fig. 2: Transit-time ultrasound Flowprobe on mesenteric (jejunal) branch (center); Laser Doppler Probe on mucosa on the right.

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Rat Mesenteric Branches: Acute Flow Measurement Cont.

After treatment of the animals with isoprenaline and superfusion of the preparations with papaverine, the mean measured flow increased to 0.666 ± 0.379 ml/min.

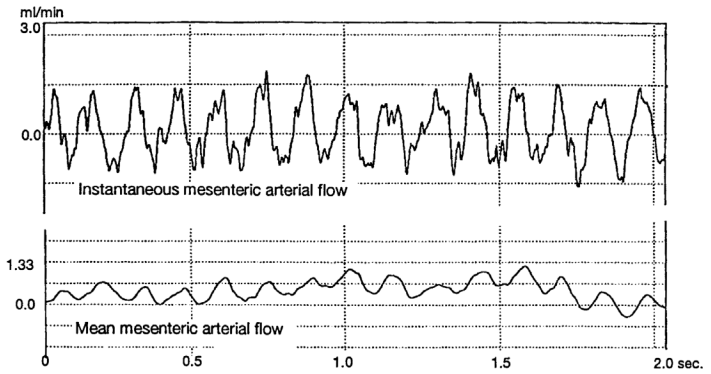

Fig. 1: Mean flow over 90 seconds was 0.494 ± 0.276 and 0.527 ± 0.233 ml/min, respectively. After treatment with isoprenaline and superfusion of the preparations with papaverine, mean flow increased to 0.666 ± 0.379 ml/min.

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Jackson EK, Herzer WA, "Angiotensin II/Prostoglandin I2 Interactions in Spontaneously Hypertensive Rats," Hypertension 1993, 22: 688-98. (344A)

Turnage RH et al, "Splanchnic PG12 Release and "No Reflow" Following Intestinal Perfusion," J Surg Res 1995; 222(1): 66-72.

Rat Femoral Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Rat
Body Weight:	200 - 400 grams
Duration:	Acute
Vessel Diameter:	
	0.7 - 0.9 mm proximal to epigastric
	0.5 - 0.6 mm distal to epigastric

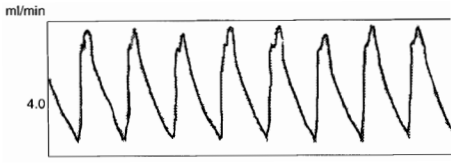
PROBE

Size:	0.7 or 1 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7PSB
	MA-1PRB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed



Flow trace courtesy of T.L. Smith, Wake Forest University School of Medicine, Winston Salem, NC.

Application

The femoral artery is a convenient site for the blood flow measurement in basic hemodynamic research as it is relatively accessible and does not require highly invasive surgery. Since access does not require opening the abdomen or the thorax, there are fewer complications from hypothermia and shock. This site is also relatively free of adipose fat.

Surgical Approach

Anaesthetize the rat with ketamine/xylamine solution (0.09 ml solution / 100 gm body weight IM thigh). Use a heating pad or heat lamp to avoid hypothermia which will reduce flow. A 0.5 ml bolus of saline placed subcutaneously every half hour is also recommended.

Note: The femoral artery tapers from 0.9 mm diameter at the iliac artery to 0.5 mm distal to the epigastric branch. Flowprobe size will depend on the chosen measurement site.

Place the rat in dorsal recumbency and visually identify the femoral vessels on the medial thigh. They should be readily visible through the shaved and prepared skin. Gently stretch the skin caudally, make a 1.5 cm incision adjacent to the femoral vessels, and release the skin so that it slides back over the vessels.

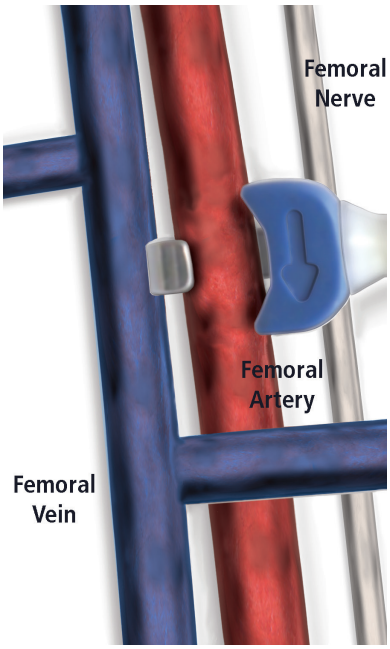


Fig. 1: Site of femoral artery in relation to femoral vein and femoral nerve.

Rat Femoral Artery: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

When the combined femoral artery, vein and nerve are exposed, carefully separate the vessels from the surrounding fascia. First pass a small curved needle completely under the vascular bundle and back out the other side, then use a fine pair of curved forceps to tease away the fascial layers. Continue this process until the vascular bundle is completely undermined and freely movable.

Separation of the vessels is often the most difficult step of the procedure. One technique is to take a pair of forceps with a very blunt tip and repeatedly open and close them in a direction parallel to the vessels. Try to make a small gap between the vessels. The vein may be identified by alternately applying pressure proximally and distally to distend and collapse the vein. When the femoral artery is isolated, place the Flowprobe around the artery. Use a micromanipulator to stabilize the Probe position on the vessel.

Remove the plunger of a 30 cc syringe and load the syringe with Surgilube gel, taking care to prevent the formation of air bubbles. Place a flexible 20 gauge catheter on the tip of the syringe. Insert the catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The gel acts as an acoustic couplant and must replace the air space. Select the test mode on the Meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of Surgilube gel or to an air bubble.

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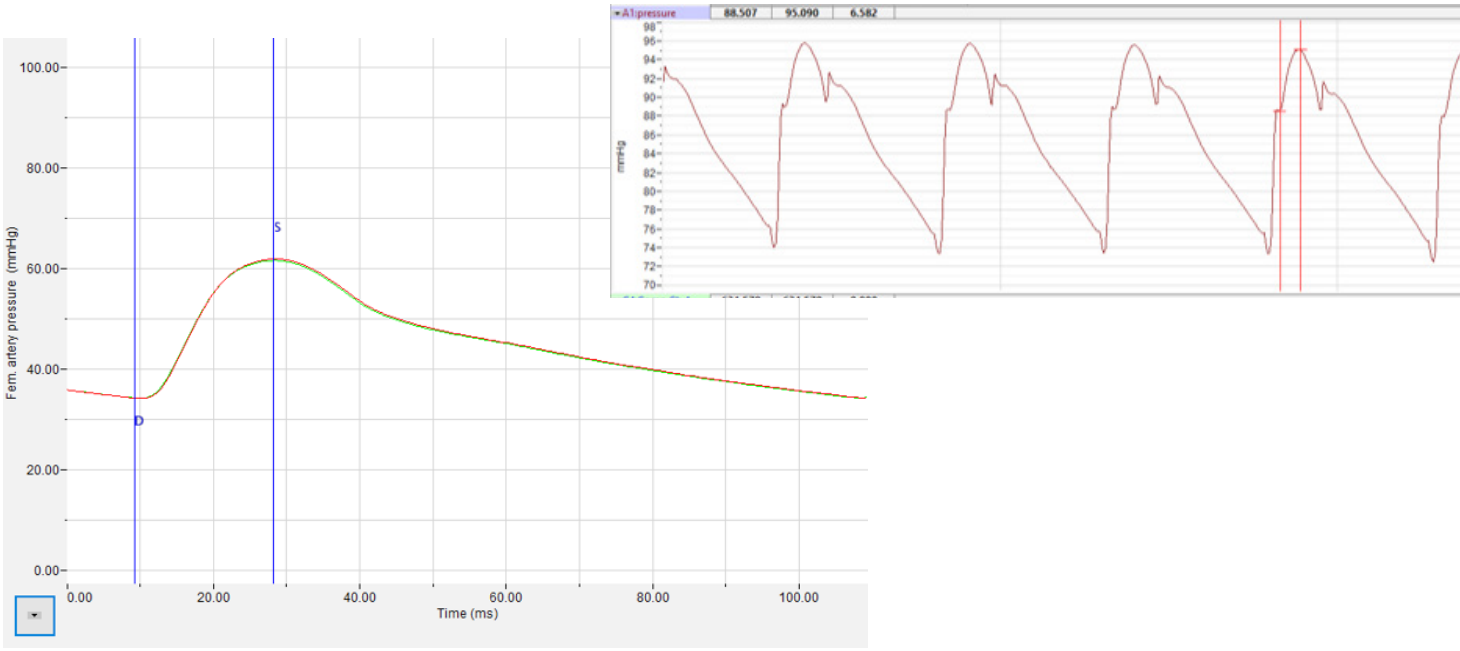
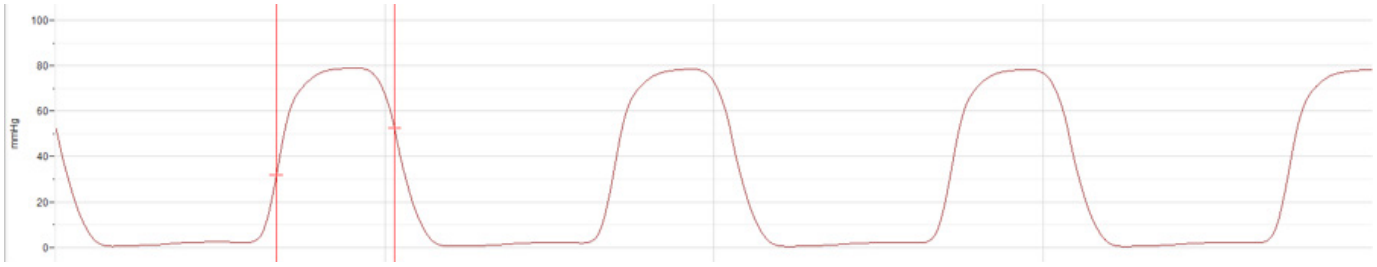
Software Set-up and Data Collection

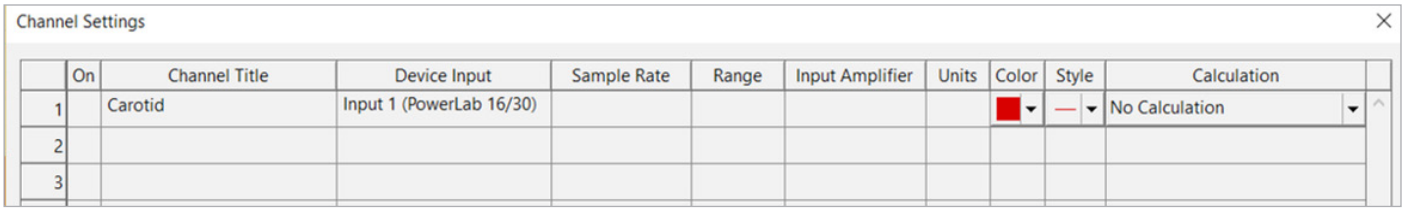




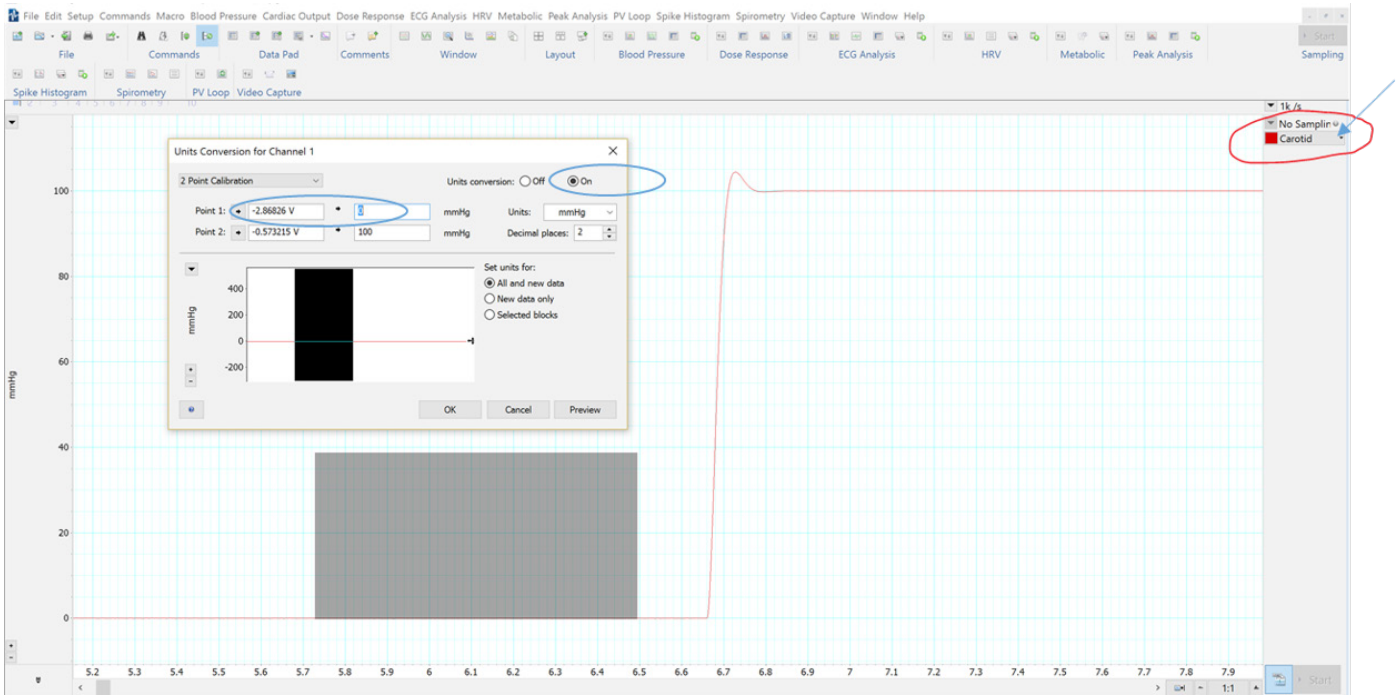
Transonic Scisense pressure and Transonic flow measurement systems both provide analog signals via BNC connections that can be collected, converted to digital and analyzed with all commonly used laboratory data acquisition systems and associated software. Since it is up to you, our customers, to choose your own DAQ, we have provided on the following pages, short documents on how to set-up a two point calibration in the most commonly used analysis software packages. This will allow a quick start in analyzing your recorded data.

Two-point Pressure Calibration Set-up with LabChart

1. Connect the control unit (SP200, SP430 or ADV500) to the pressure catheter and data acquisition unit.
2. Connect the data acquisition unit to computer and start LabChart software.
3. Select setup and then Channel setting, then click **OK**.
4. Then fill the Channel Title and other blank boxes.

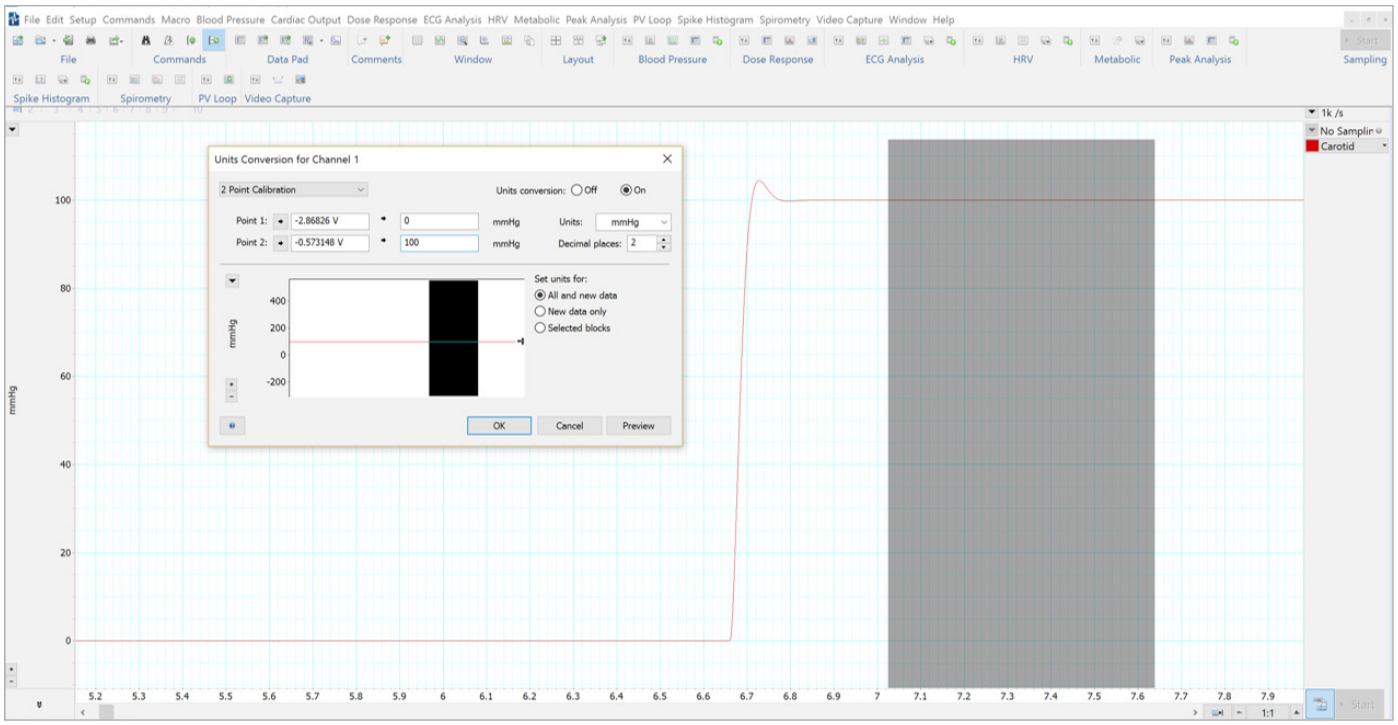


5. On the pressure control unit, select 0 mmHg to send a voltage signal and record about 2 seconds of data.
6. Then select 100 mmHg on the control unit to send a second voltage signal and record for about 2 seconds.
7. Click on arrow close to channel name (Carotid in this example) and select unit conversions.
8. The unit conversion dialog box will appear.
9. Using the mouse, select the voltage signal that was recorded using the zero setting on the pressure control box (selected black in the dialog box, grey on the trace display in the image below).
10. Point 1: will automatically display the average voltage for the selected area. Then you click to associate this voltage (-2.86826V) to set 0 mmHg from pressure control unit. The unit conversion radio button should be on.



Two-point Pressure Calibration Set-up with LabChart Cont.

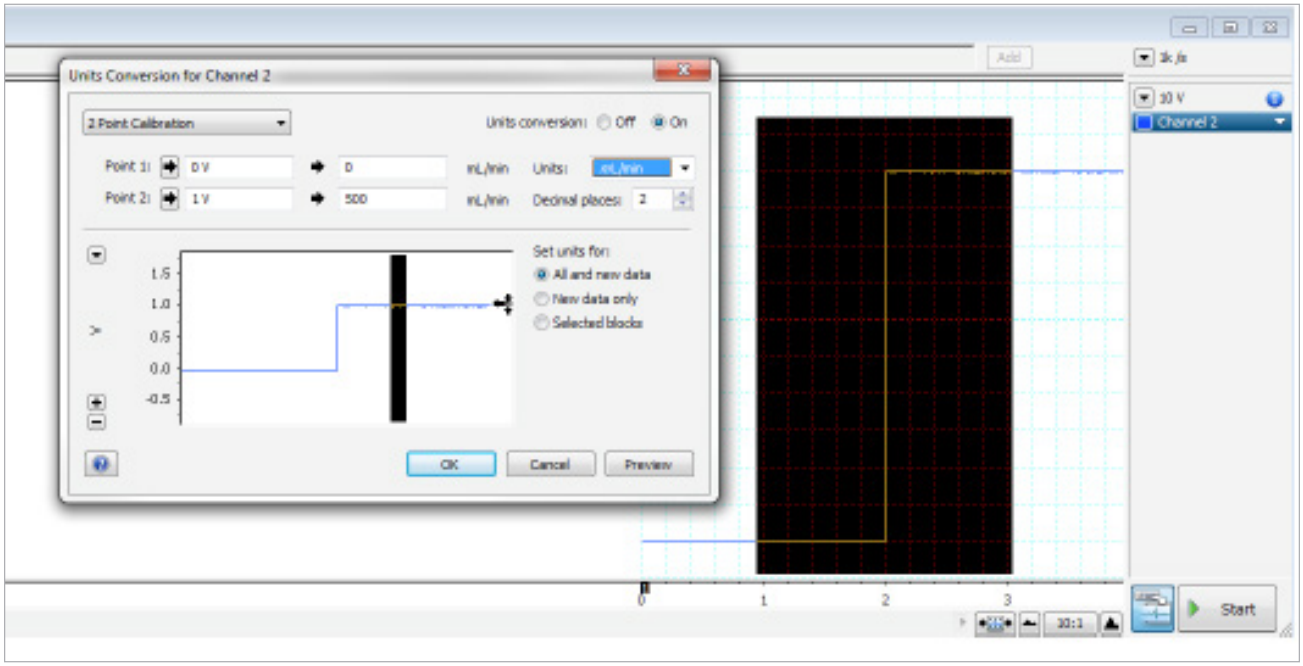
11. Next, select the voltage signal that was recorded using 100 mmHg (selected black in the dialog box, grey on the trace display in the image below).
12. Point 2: will automatically display the average voltage for selected area. Then you click to associate this voltage (-0.573140V) to set 100 mmHg from pressure control unit. The unit conversion radio button should be on.
13. Select decimal place to 2, and set units for All and new data. Then click **OK.**



Two-point Volume Flow Calibration Set-up with LabChart

To start recording blood flow measurements you need to set up LabChart to properly process the voltage signal coming from the perivascular flow meter. After connecting the flow probe, follow the steps below:

1. Put the flow meter in **TEST MODE**.
2. Observe the signal quality on the top left corner of the flow meter. The light bars on the signal quality indicator should indicate a minimum of 3 bars, with 5 being optimal. Low signal may indicate a need for acoustic coupling gel.
3. Start recording in LabChart by pressing the **Start** button on the top right corner of the computer screen.
4. Press the **MODE** button on the flow meter to change the mode to **ZERO**. The analog needle will deflect to 0 Volts. This will output a 0V signal into LabChart.
5. Subsequently, press the **MODE** button to change the mode to **SCALE**. The flow meter will put out 1V to the PowerLab and the display will show the flow value in mL/min or L/min that corresponds to 1V for this specific probe size.
6. Press the **MODE** button once more to switch to **MEASURE** in order to observe the measured flow rate in the system.
7. Stop recording in LabChart.
8. Select area with the 0V and 1V data.
9. Go into the channel menu for the flow channel on the right side of the computer screen and choose Units Conversion after a mouse click.
10. Highlight a portion of the 0V area of the data, assign this to Point 1 (see image below) and enter 0 in the block to the right. Highlight a portion of the 1V area of the data, assign this to Point 2 (see image below) and enter the 1 volt scale value for the probe that was displayed in the SCALE mode in the block to the right.



11. Assign the appropriate units (mL/min or L/min).
12. Select **OK**.

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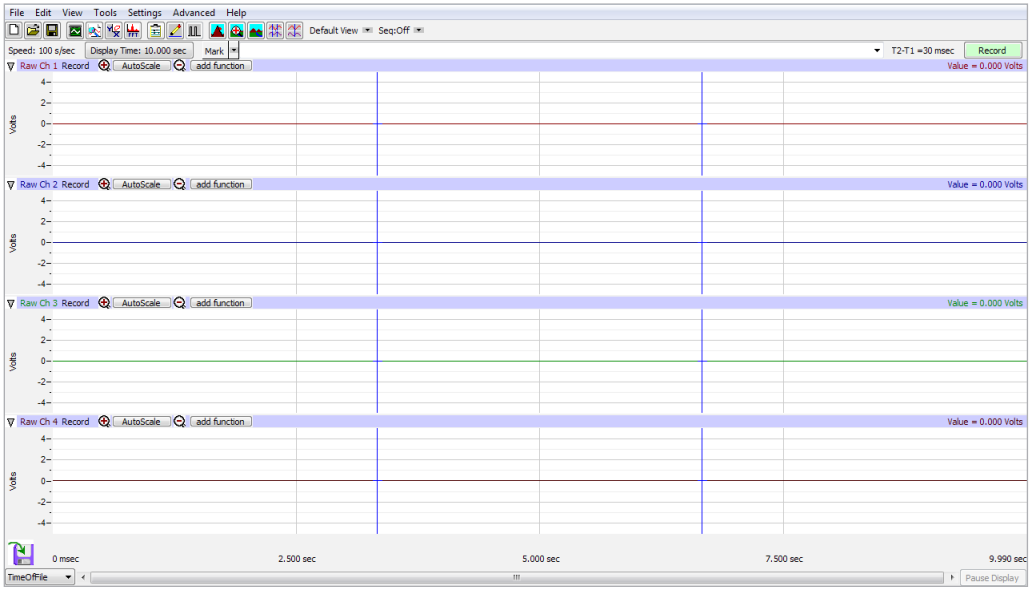
How to Create a Blood Pressure Configuration File in LabScribe2 Software

The following document outlines the essential and suggested steps for creating the ideal blood pressure configuration file for blood pressure recordings using the SP200 control unit. In this file researchers will create a configuration for proper recording of vascular or chamber blood pressure.

Refer to the "SP200 Quick Start Guide" and "SP200 User Manual" for specific hardware operation steps. Before starting, ensure that your iWorx data recording hardware is properly connected to your PC or Laptop and recognized. The following Guide shows LabScribe2 used with a 4-channel Data Acquisition system, Model IX-404. For other hardware, essential steps remain the same; however, for Model IX-228 remember that Channels 1 and 2 are hard-wired for EKG recordings via the supplied AAMI cable. Therefore, Channels 3-10 are for suitable for recording from other analog devices.

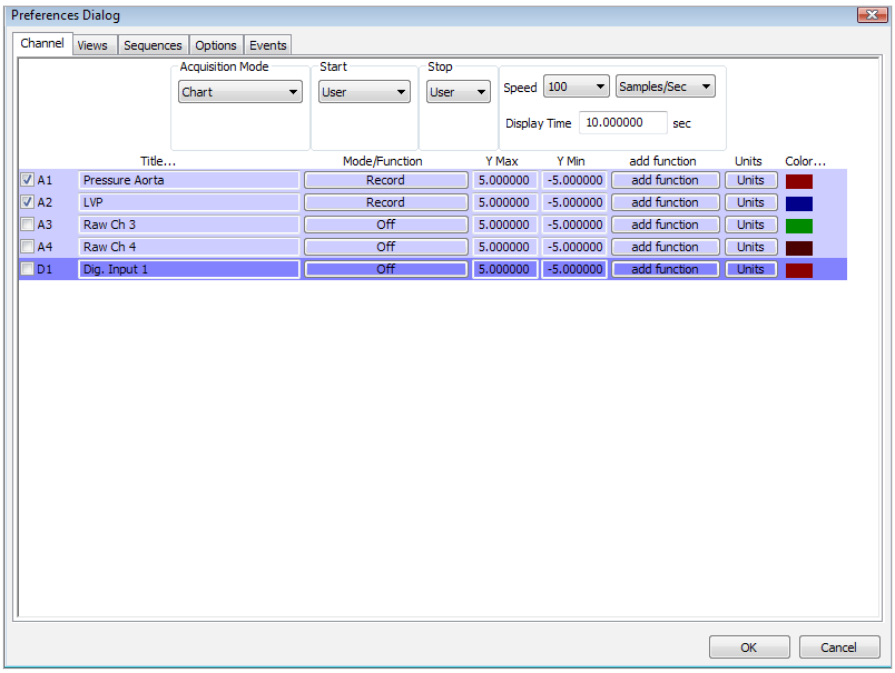
1. Open up LabScribe2 Software – upon start-up a dialog box will appear to confirm recognition of the recording hardware. In the event the dialog box does not appear, select **Tools > Find Hardware** to recall the hardware search. If hardware recognition continues to fail shut down LabScribe2 and check that the USB port is properly working, that all connections between the hardware and computer are secure, and that all iWorx hardware drivers are correctly installed (see iWorx User Manuals for information).
2. After selecting **OK**, the Main Window will be accessible. This guide assumes the configuration is being created from a Default template: remember LabScribe2 always opens using the previously used settings. Therefore, to remove previously used settings and return to a Default template, select **Settings > Default Setting**. In the Default Setting four channels will appear with the titles "Raw Ch1" through "Raw Ch4". All channels will be presented in Voltage Scale.



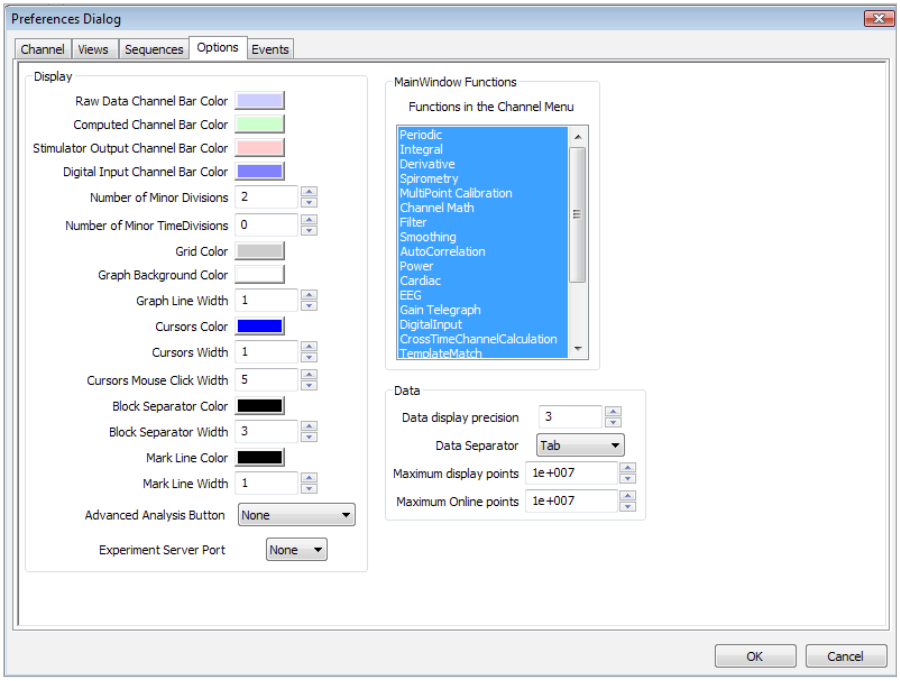
Main Screen with Default Settings.

How to Create Pressure File in LabScribe Cont.

3. Select **Edit > Preferences**. The Preferences Dialog box will appear with five internal tabs: Channel, Views, Sequences, Options, and Events. Starting with the **Channel Tab**, input the following:
   a. Speed: this setting determines how many samples/second the hardware recorder will measure the incoming analog signals. It is very important to sample data at the appropriate speed to ensure optimal resolution and accuracy.
      i. For Rodent blood pressure Studies: 1000 Samples/Second
      ii. For Rabbit blood pressure Studies: 500 Samples/Second
      iii. For Large Animal blood pressure Studies: 200-500 Samples/Second (200 is adequate assuming Heart Rate is below 100 BPM)
   b. Input Channels: ensure that the correct number of channels are selected for Recording (a check-mark will appear in the box for each channel). Enter Titles for each Channel: it is suggested to use descriptive titles: Pressure Aorta (vessel), LVP (RVP) or similar.
   c. Views, Sequences, Options, and Events: collectively, these tabs hold additional preferences non-essential for proper data recording. Briefly, here is a description for each:
      i. Views: allows additional "Views" to be created where specific Raw Channels, Function Channels, Voltmeter Panel, and Online XY options can be arranged to the Researchers Preference.



Channel Tab of Preferences Dialog Box. Only select those channels which will be used by clicking or un-clicking the check boxes along the left. This shows two channels for a dual pressure system.



Additional Preferences can be adjusted as desired.

How to Create Pressure File in LabScribe Cont.

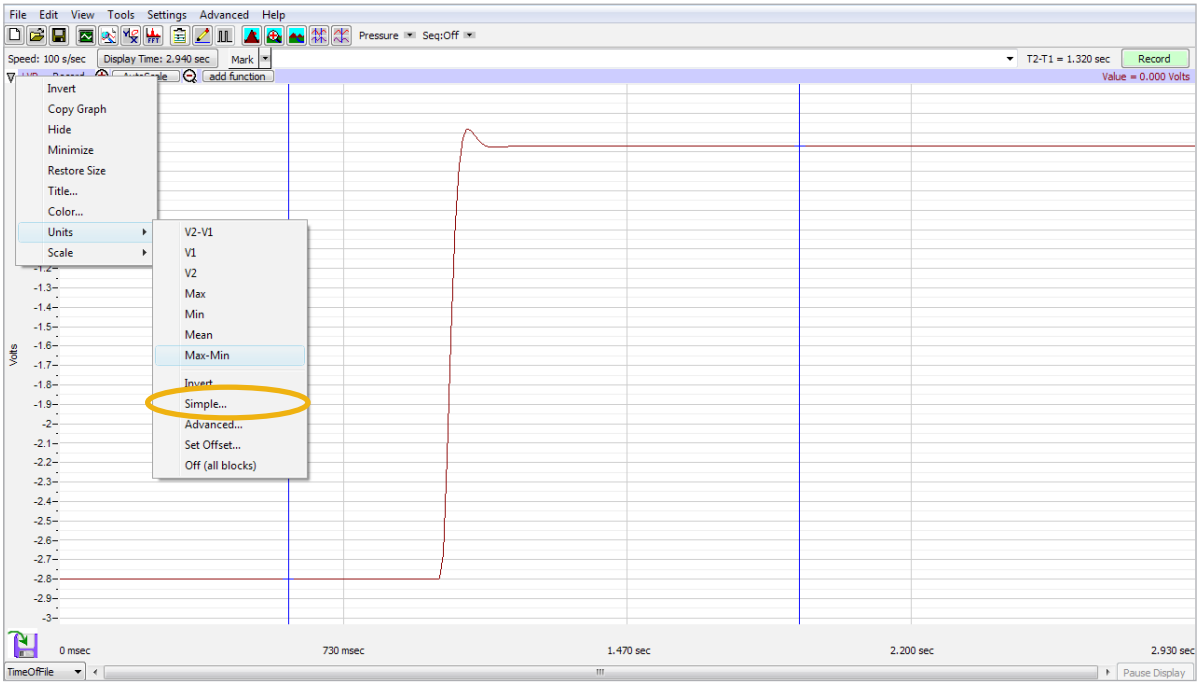
ii. Sequences: allows a "sequence" to be written. In general, an event or action will start a sequence, following a series of actions will commence (ie. start recording, make a mark, stop recording). Sequences are used in combination with Events, and in general do not suit blood pressure protocols.

iii. Options: includes all preferences for Display on the Main Window, Data point display and the precision of this data. TIP: take notice of the "Main Window Functions" section. Only the blue highlighted items will appear in the drop-down list when "add function" buttons are selected in the Main Window.

iv. Events: allows an "event" to be written. Events work hand-in-hand with Sequences. An event is an occurrence that can be measured or recognized by the software on a specific channel: once these specific events are entered (ie. pressure recording reaches a peak value of 120 mmHg) a sequence can start (ie. start recording data). Again, this type of preference or function is not suited for blood pressure research.

4. Click **OK** in the Preference Dialog to return to the Main Window. Title(s) should now appear for each raw input, and the correct sampling speed will be applied (see top-left corner). The next step is to calibrate the raw input channels in their appropriate scale (mmHg). On the SP200 (with a catheter connected), push the light blue button (0 mmHg) and zero mmHg or low cal signals will be output from the system displayed on the screen. Hit the RECORD button in LabScribe2 (green and at right hand corner of computer screen) to start recording. After collecting Low calibration signals, push light blue button (100 mmHg) to output 100 mmHg high calibration signals.

Channel	Low Cal (V)	High Cal (V)
Pressure	-2.86 ± 0.01	-0.57 ± 0.01



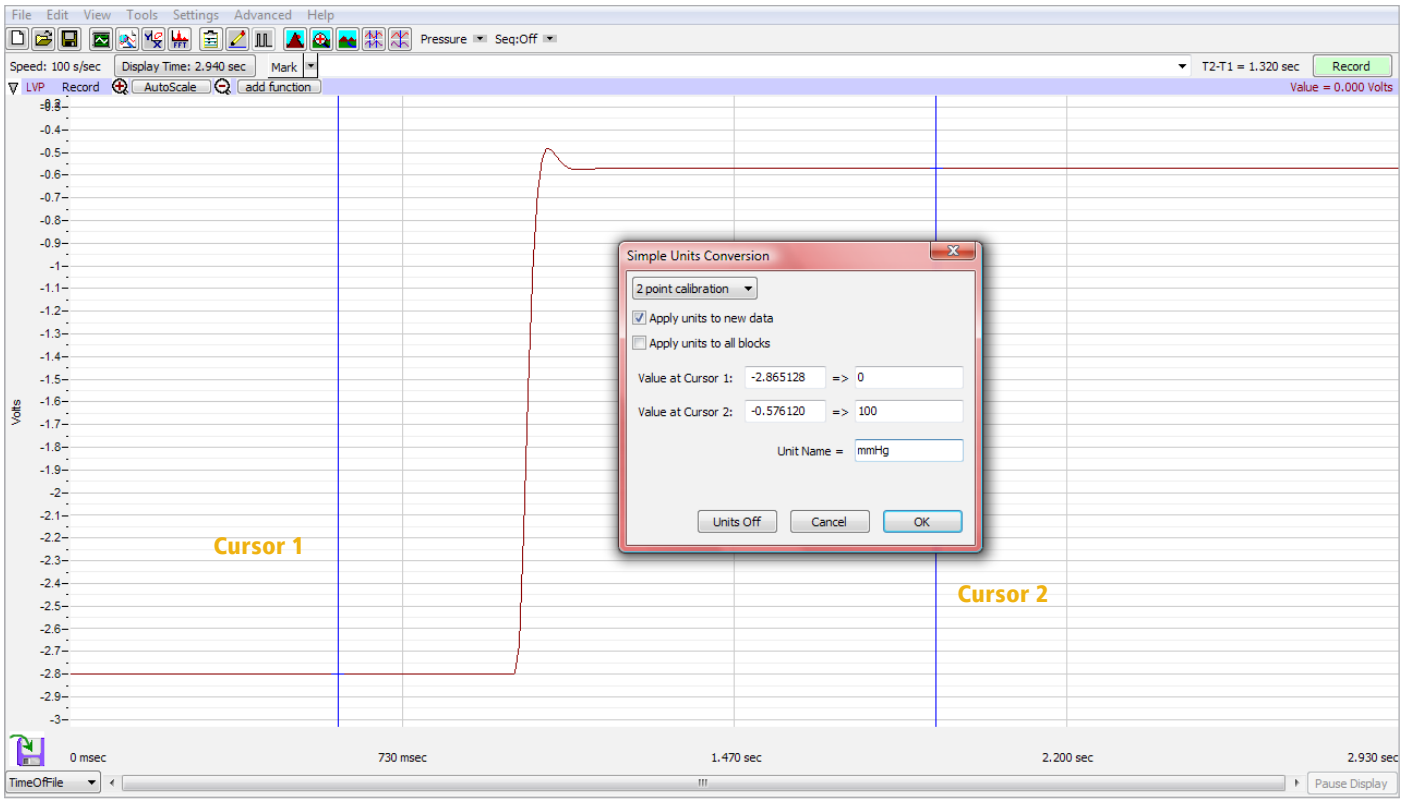
Press 0 mmHg and then 100 mmHg on the SP200 to send low and high calibration signals. Open the Simple Units Conversion menu to convert from voltage to pressure.

How to Create Pressure File in LabScribe Cont.

5. After successfully recording both Low and High calibration signals on channel(s), press the STOP button to end recording. Using the two vertical blue cursors, set the left cursor to intersect the Low output values, and the right cursor to intersect the High output values.

NOTE: if only one vertical cursor is available, click on the “Double Cursors” icon that is located in the top menu bar.

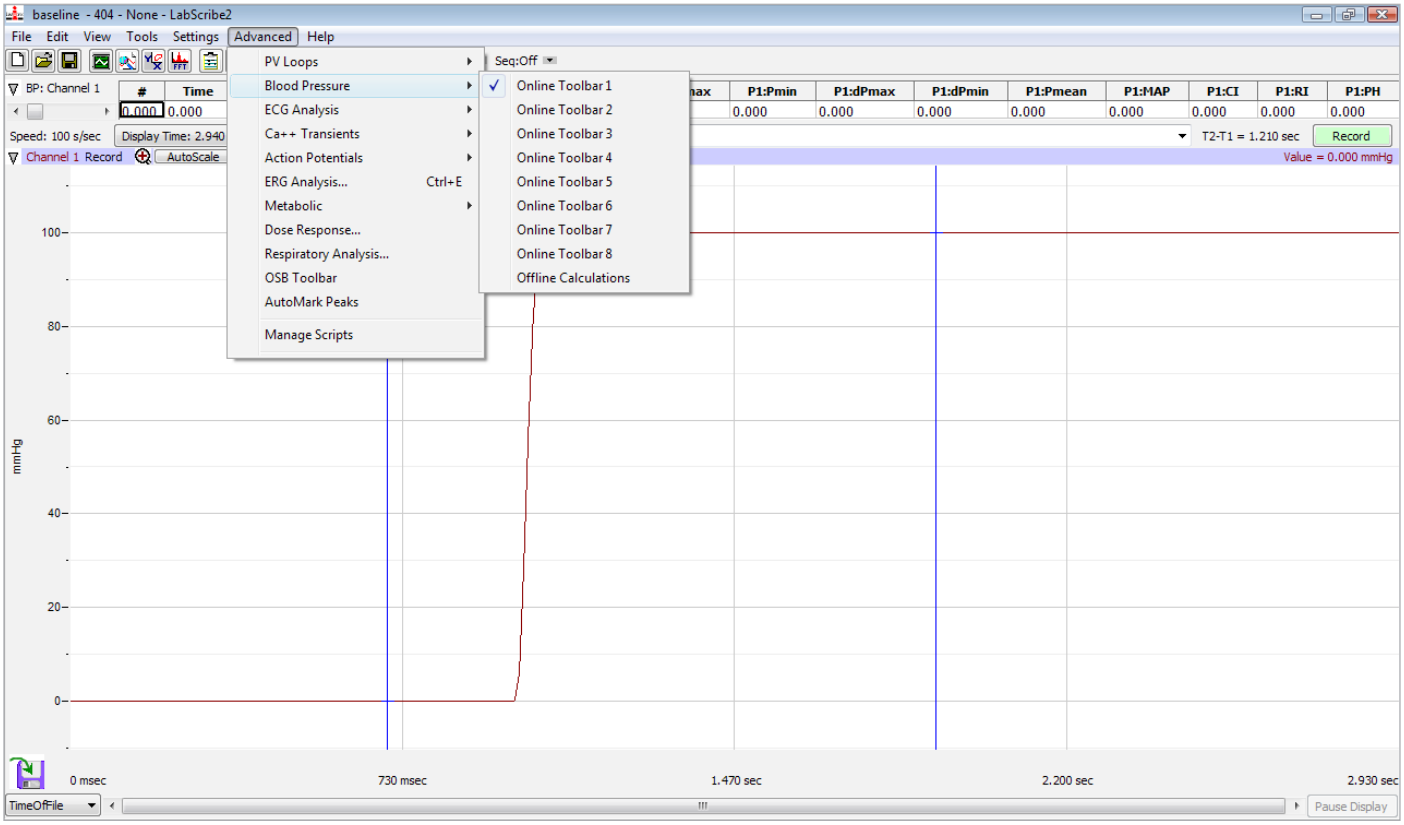
6. After correctly placing the vertical cursors, select the Pressure Channel drop-down Menu (inverse triangle beside channel title). From this menu, select **UNITS > SIMPLE**. The Simple Units Conversion dialog box will appear. Conversion is from raw voltage into mmHg. Keep the default settings (2 point calibration and Apply units to new data). NOTE: to apply calibration settings to previously recorded data, select the radio button for Apply units to all blocks. Value at Cursor 1 will show the voltage measured at the left cursor (in this case the Low Pressure calibration value), while Value at Cursor 2 will show the voltage measured at the right cursor (in this case the High Pressure calibration value).
7. Enter the corresponding Low and High calibration values (i.e. 0 and 100) and input the Unit Name for that particular channel (i.e. mmHg). Repeat this process for the second pressure channel (if used).



Use the values from the two cursors to convert the signal from voltage to pressure.

How to Create Pressure File in LabScribe Cont.

8. Next, select **ADVANCED > ONLINE TOOLBAR 1** to activate the Online blood pressure calculations module. After the Online blood pressure calculation bar appears at the top of the screen, select the BP LVP drop-down menu (inverse triangle to the left the BP LVP calculations bar) and select **SETUP**. Note: all modules are purchased separately from Iworx including Blood Pressure module software.



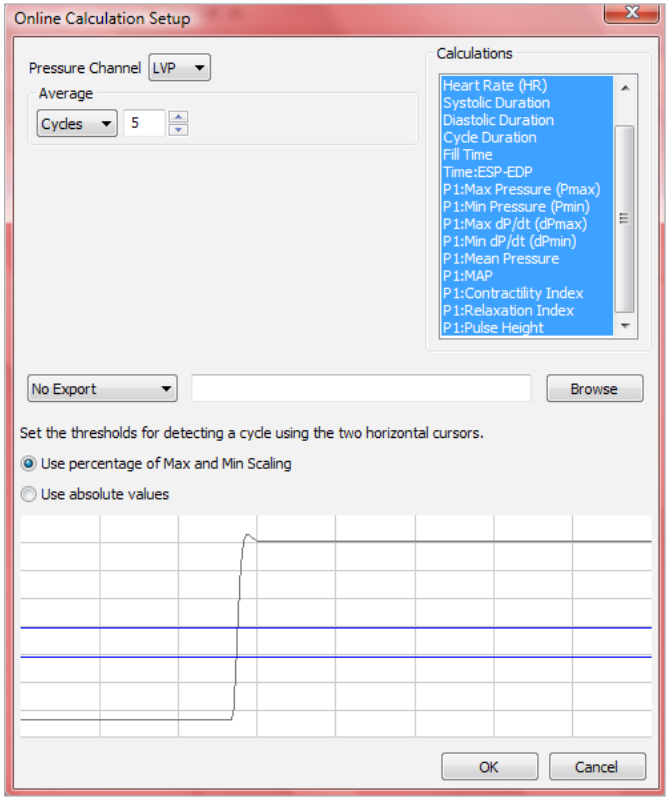
Open the Online Toolbar 1 Menu

9. Upon selecting the Setup feature, the Online Toolbar 1 Dialog Box will appear. The selections in this area are very important to ensure that Online calculations will run correctly. Ensure these details are correct:
   a. Pressure Channel: select the main Pressure Channel
   b. Cycles to Average: this changes the online table from beat-by-beat (Cycles to Average = 1) to display mean data based on a number of qualified cycles. We suggest 10 beats for rodent studies and 5 beats for large animal.
   c. Calculations: the default setting includes all hemodynamic parameters, but all of these parameters are not required for viewing in real-time.
   d. Threshold Setting: it is ideal to leave the Threshold Setting (the function that determines a cycle by analyzing the pressure waveform) as a Percentage of Max and Min Scaling. In addition, drag the top horizontal threshold cursor DOWN to both lower the Max pressure value and reduce the margin between the bottom horizontal cursor. This will ensure the software effectively registers each beat from the LV pressure waveform.

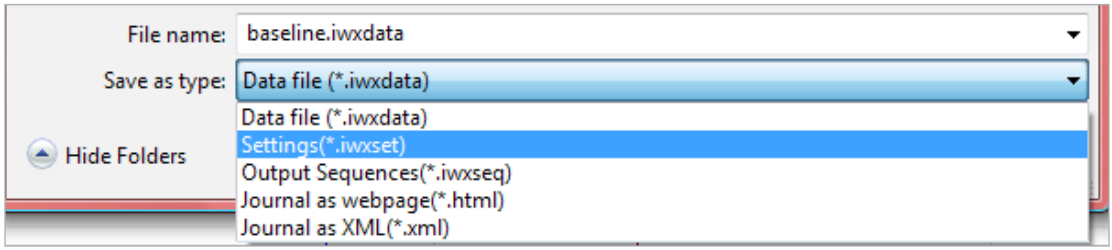
At this point a standard blood pressure Settings File is complete.

How to Create Pressure File in LabScribe Cont.

Select **FILE > SAVE AS.** dialog box will appear. Select the desired location to save the settings file. Next, change the Save as type: to Settings (*.iwxset). Give the settings file an appropriate name (i.e. Rat Blood Pressure Template), and click SAVE.



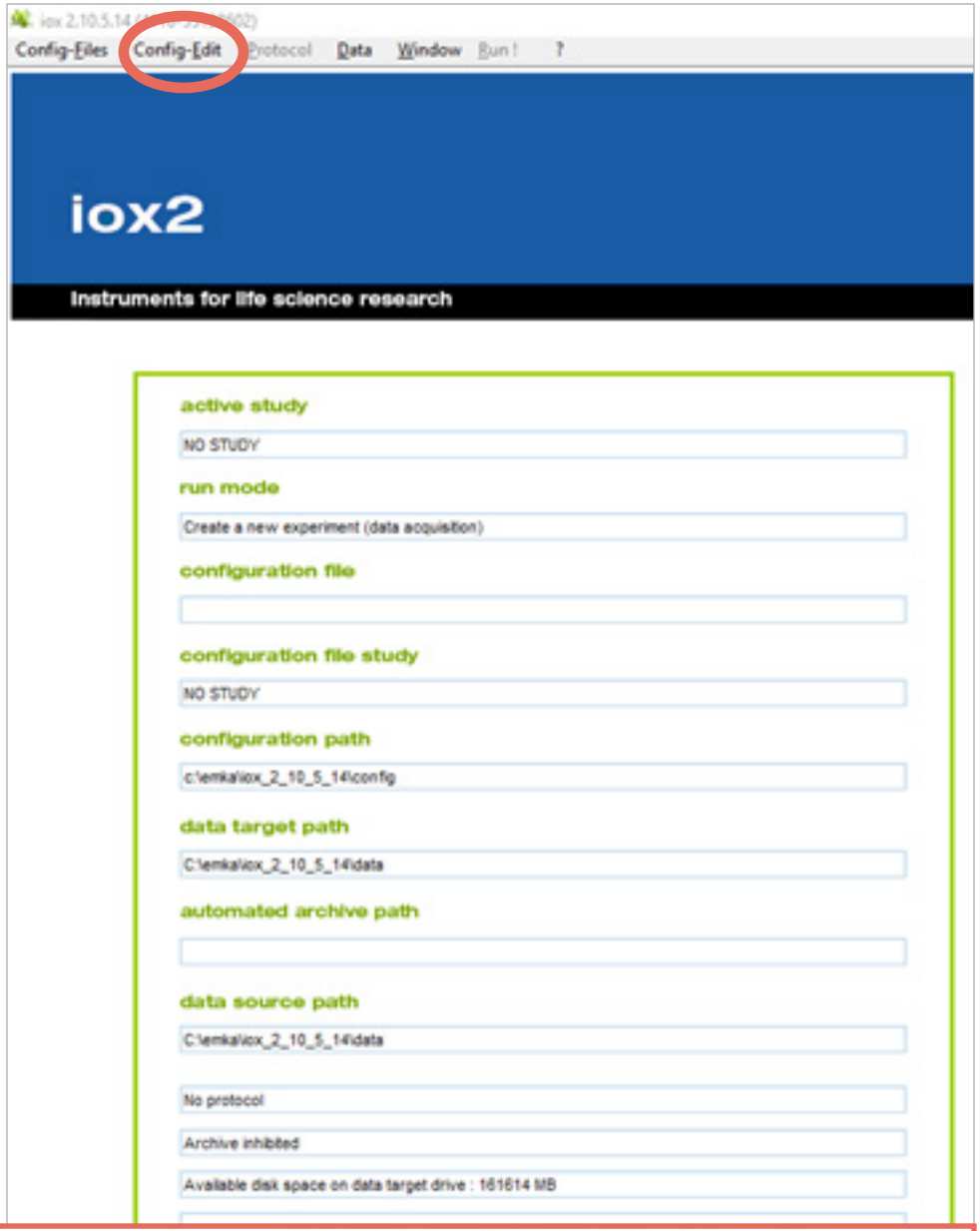
Adjust the Online Calculation Setup values

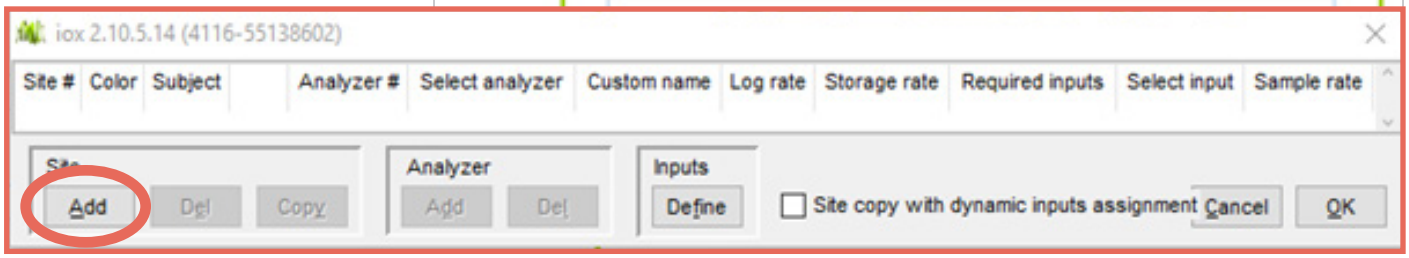


Save as type: Settings

How to Create a Pressure Configuration File in iox

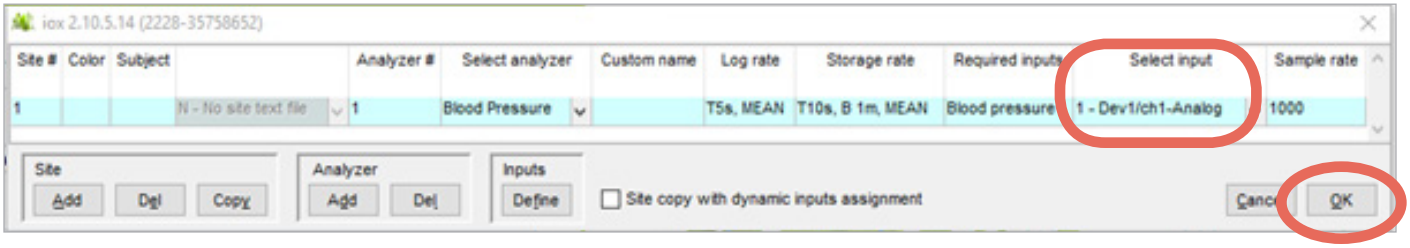
1. Connect control unit to pressure catheter and data acquisition unit (usbACQ)
2. Connect data acquisition unit to computer and start iox software
3. Click on **Config-Edit** and then Inputs / Sites / Analyzer…
4. Click on **Add**



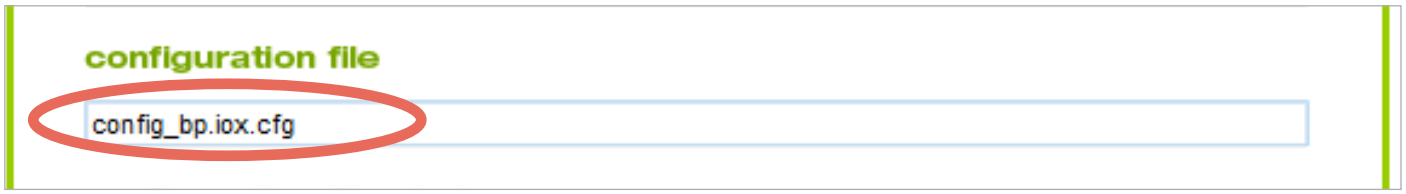


How to Create a Pressure Configuration File in iox Cont.

5. In the column “Select analyzer”, click on **Blood Pressure**.
6. In the column « Select input », ensure that the proper channel of the usbACQ is chosen (Channel 1 by default).
7. In the Inputs / Sites / Analyzer… click on **OK**.

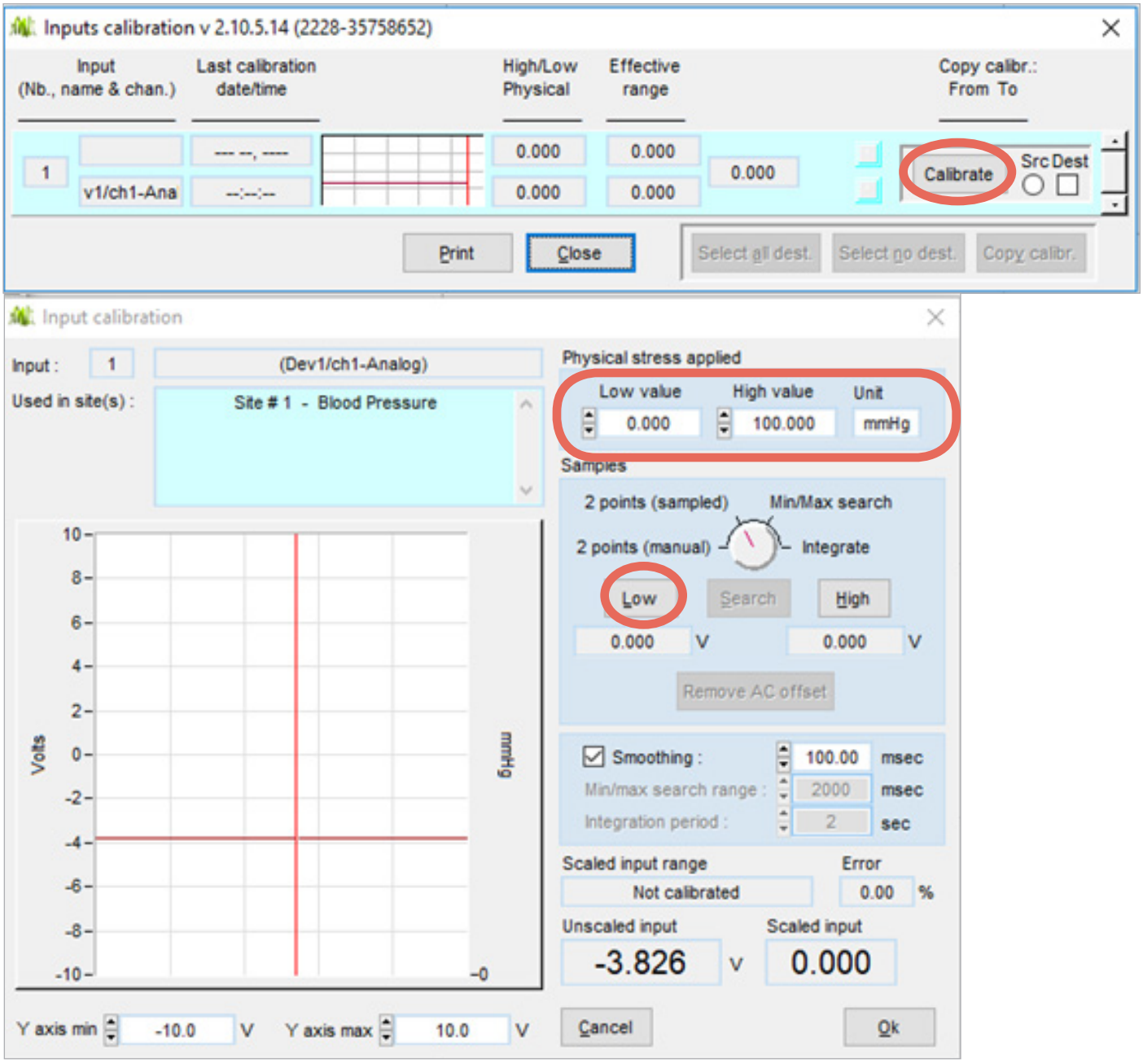


8. In the main panel, click on **Config-Files**, then Save as and give a name to your configuration. Save it and its name should appear in the main panel of iox.



Two-point Pressure Calibration Set-up with iox

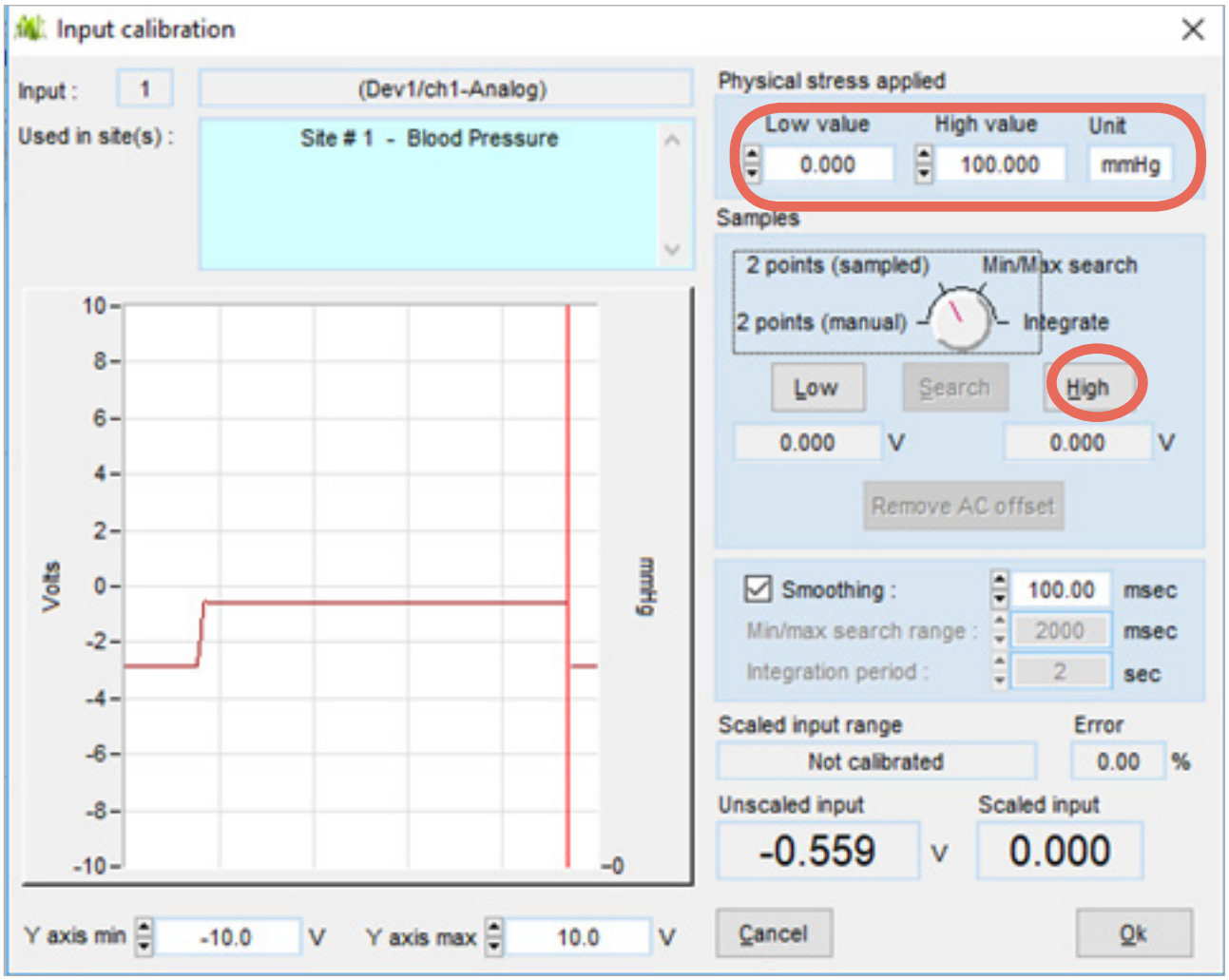
1. Once the configuration is prepared, click on **Run**
2. Give a name to your subject and click on **Run** again
3. Click on **Tuning** in the upper taskbar and then **Calibrate**
4. Send low voltage signal via the control unit (first option in System Settings on the SP200)
5. In the Inputs calibration panel, click on **Calibrate**.



6. Insert the unit, the low and high values in the corresponding fields. Click on **Low**.

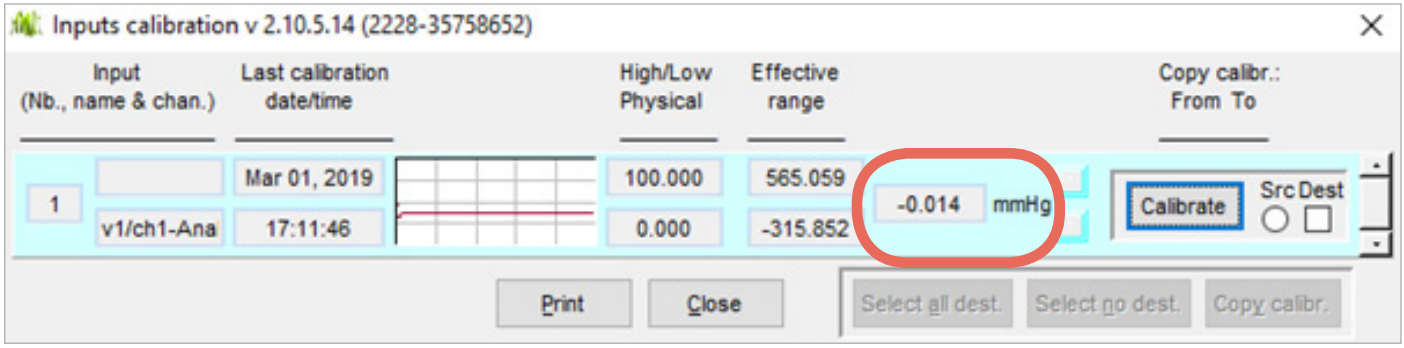
Two-point Pressure Calibration Set-up with iox Cont.

7. Send high voltage signal via the control unit and click on **High** in the input calibration screen.
8. Click on **Ok**.



Two-point Pressure Calibration Set-up with iox Cont.

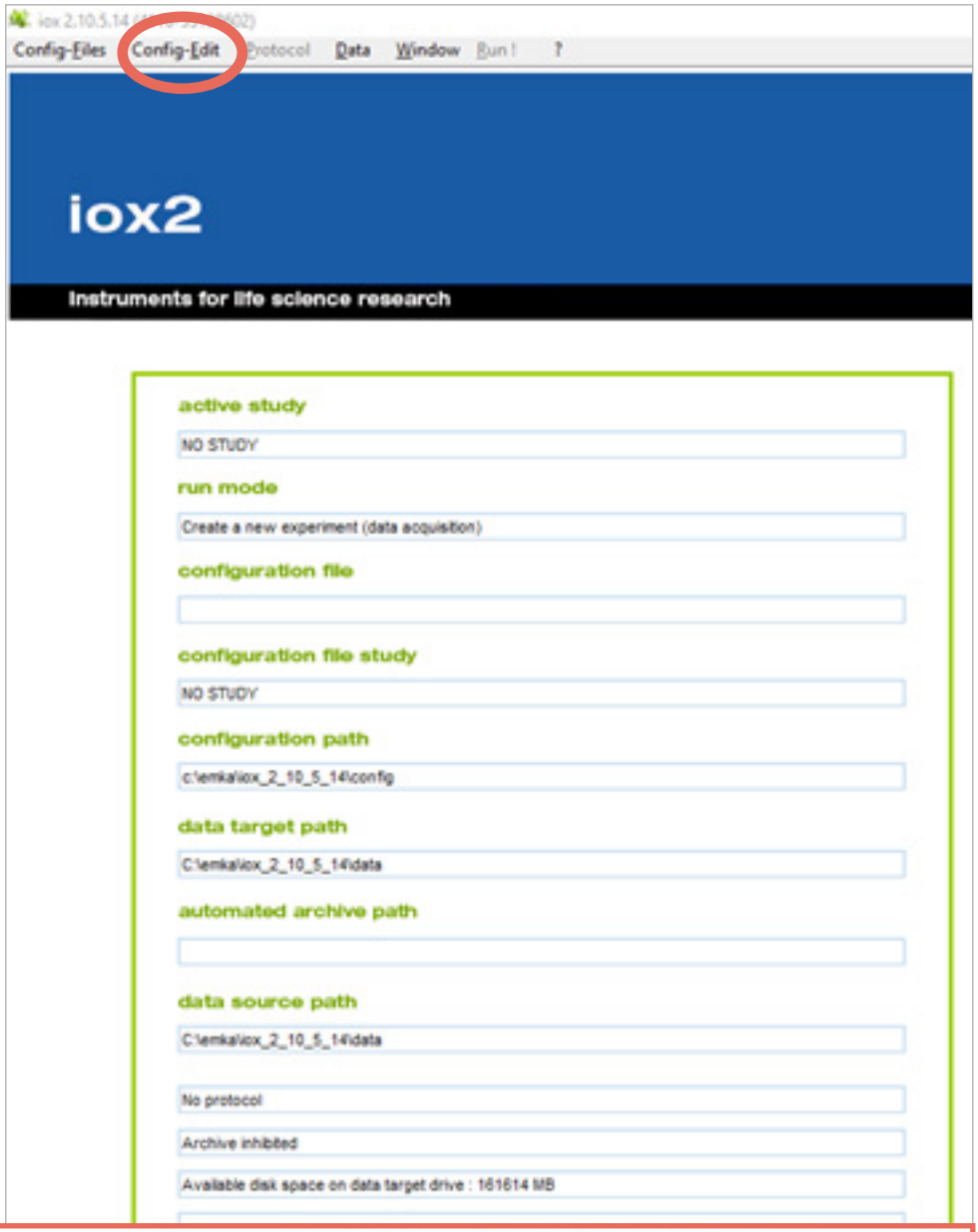
9. Once this is done, you can check that the calibration is effective. First, send low voltage signal, and check that the values are all close to zero.

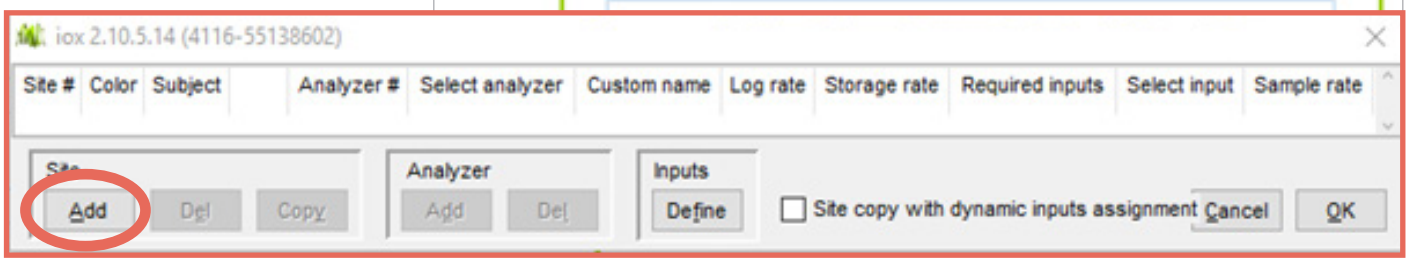


10. Then send high voltage signal and check that the values are close to the ones sent by the control units.
11. Save the calibration.

How to Create a Transit-time Flow Configuration File in iox

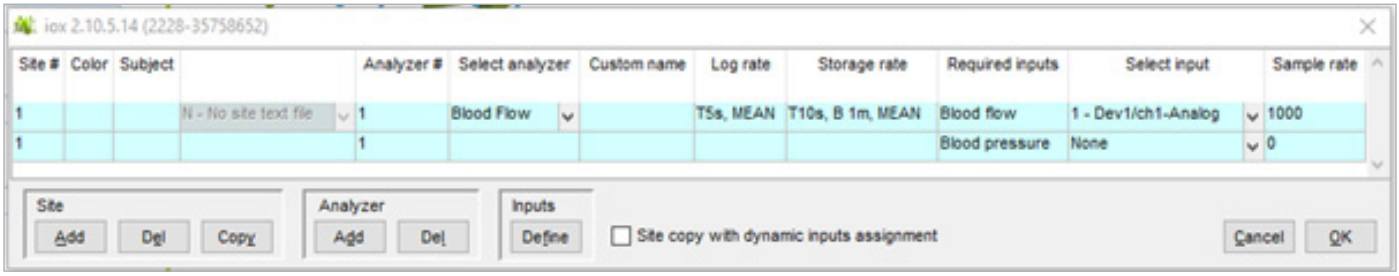
1. Connect the flowmeter with flowprobe to the data acquisition unit (usbACQ)
2. Connect data acquisition unit to computer and start iox software
3. Click on **Config-Edit** and then Inputs / Sites / Analyzer…
4. Click on **Add**



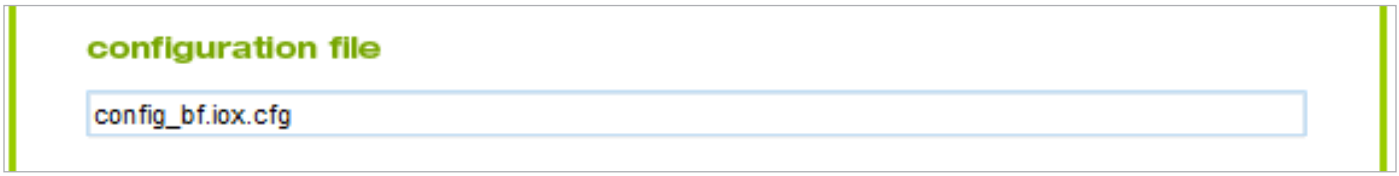


How to Create a TT Flow Configuration File in iox Cont.

5. In the column « Select analyzer », click on Blood Flow.
6. In the column « Select input », ensure that the proper channel of the usbACQ is chosen (Channel 1 by default). If a blood pressure signal is recorded at the same time, select the proper channel. If not, set this channel as None.

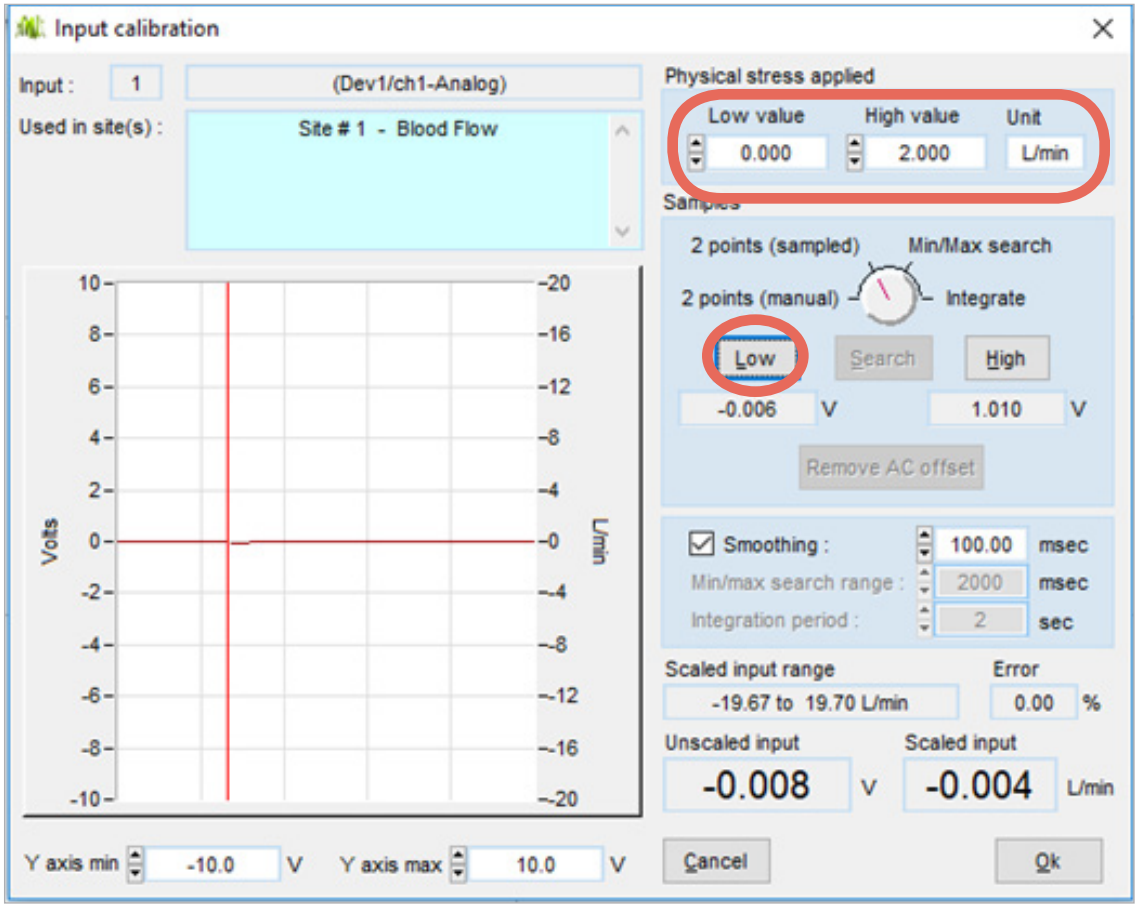


7. In the Inputs / Sites / Analyzer… click OK.
8. 9. In the main panel, click on Config-Files, then Save as and give a name to your configuration. Save it and its name should appear in the main panel of iox.



Two-point Transit-time Flow Calibration Set-up with iox

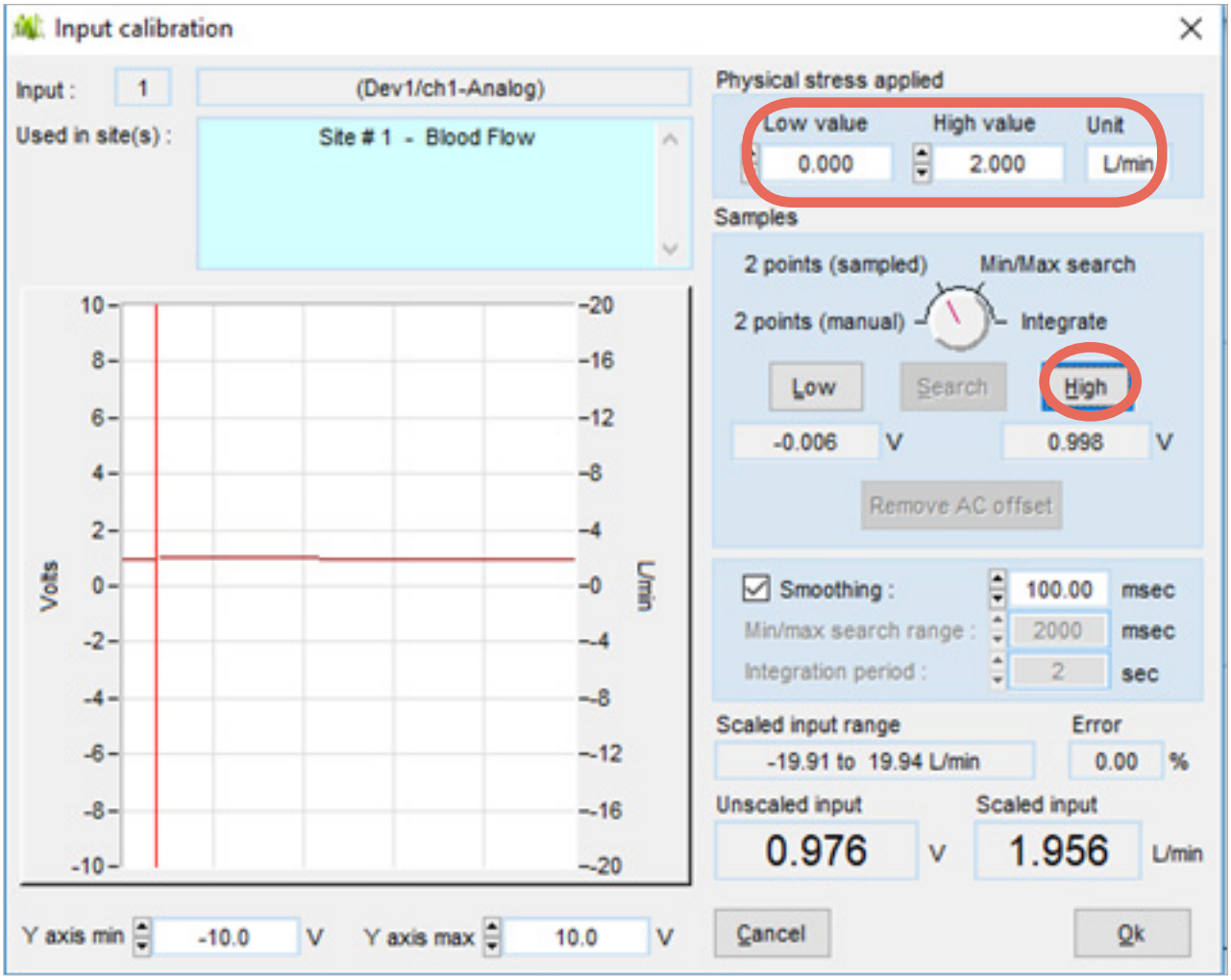
1. Once the configuration is prepared, click on **Run**
2. Give a name to your subject and click on **Run** again
3. Click on **Tuning** in the upper taskbar and then **Calibrate**
4. Send low voltage signal via the flowmeter (select **ZERO** by MODE button)
5. In the Inputs calibration panel, click on **Calibrate**.



6. Insert the unit, the low and high scale values in the corresponding fields (given by the flow meter box when you click on Zero and Scale). Click on **Low** once the signal is stabilized.

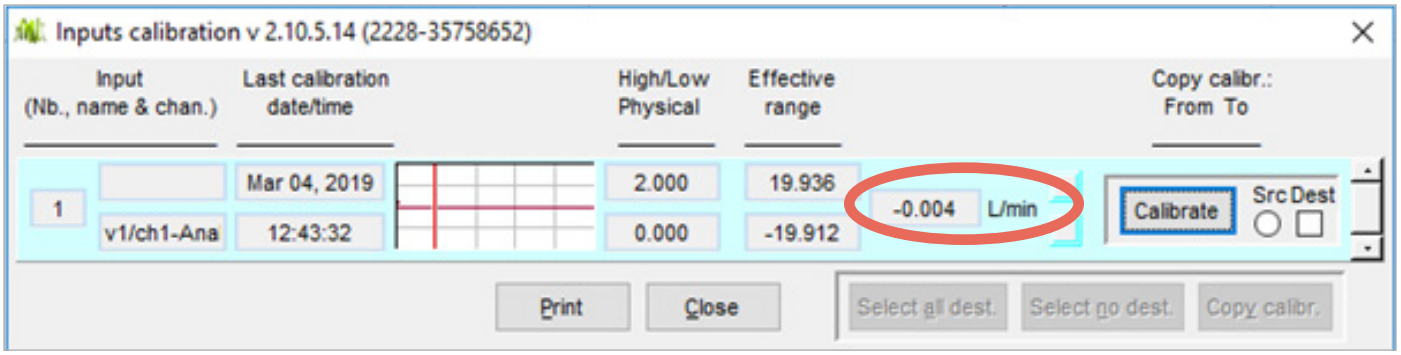
Two-point TT Flow Calibration Set-up with iox Cont.

7. Select **Scale** on the flowmeter (by the MODE button to output 1 volt signal). Click on **High** in the input calibration screen once the signal is stabilized. The high scale value is displayed on the digital display of the flowmeter.
8. Click on **Ok**.

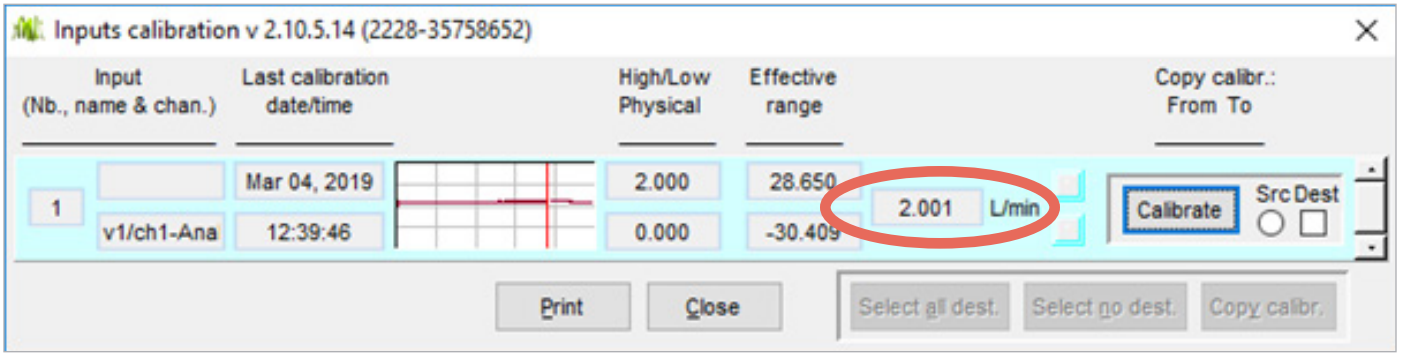


Two-point TT Flow Calibration Set-up with iox Cont.

9. Once this is done, you can check that the calibration is effective. First, select **Zero** on the flowmeter, and check that the value is close to zero.



10. Then select **Scale** and check that the value is close to the one indicated by the control units. (2L/min here)
11. Save the calibration.



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