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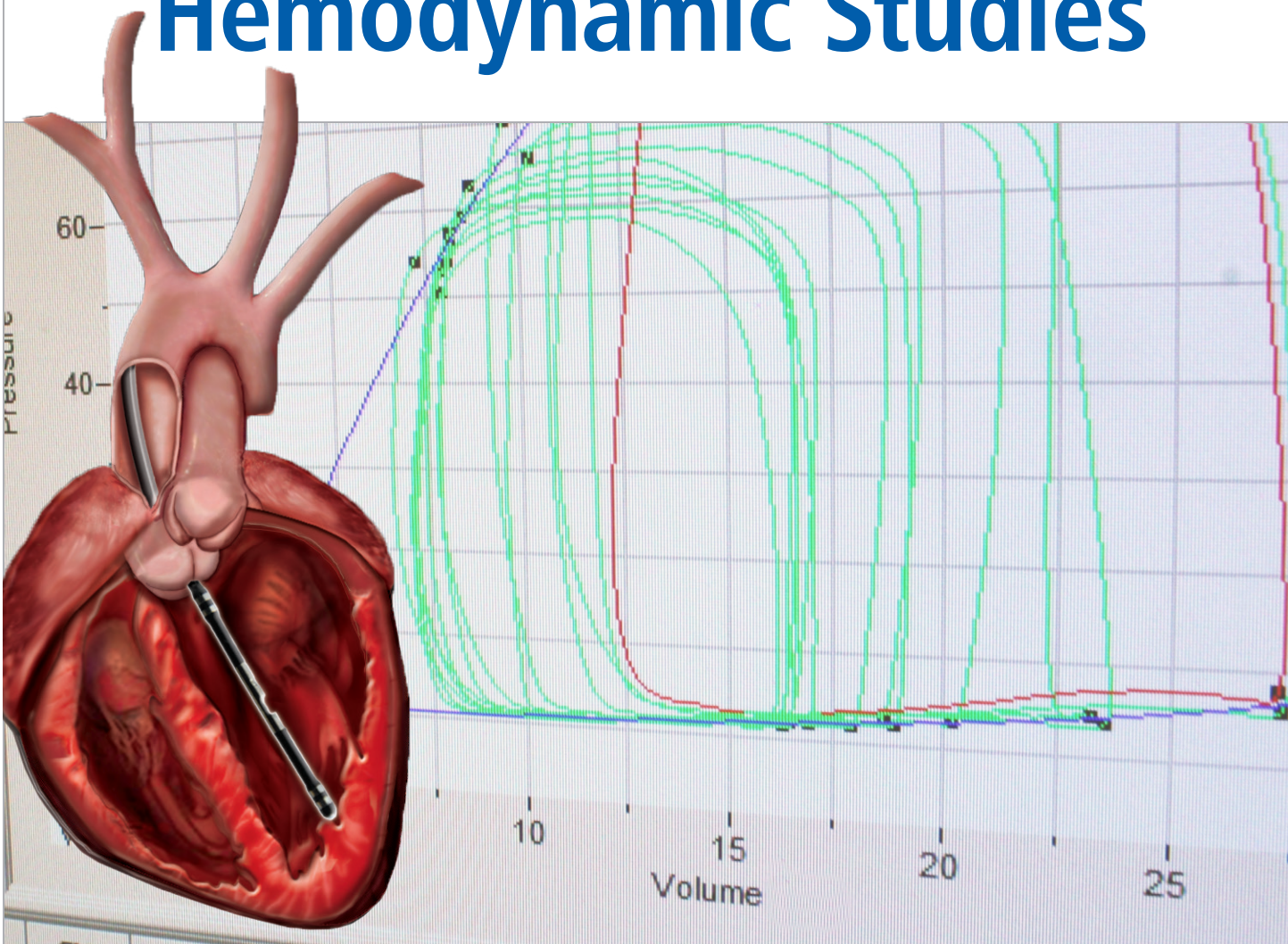
THE MEASURE OF BETTER RESULTS.

Tools & Techniques for Pressure-Volume Hemodynamic Studies



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This workbook presents protocols, tools, tips of the trade and application support for successful hemodynamic measurements using pressure-volume technology.

Within this workbook you will find detailed information reviewing all aspects of Pressure-Volume (PV) research. First we will review the technology we employ – how it works, what benefits you can expect – as well as the parameters you can measure with it. Next you will find specific equipment information, best practices for usage and care, anesthesia guidelines, aseptic techniques and more. Following this we will review several relevant applications that benefit from PV interrogation – some of these applications may be new to you. Following this application review, we jump into a detailed protocol review of the surgical steps available to perform these measures in mice, rats and large animals. Finally, you will find a quick look into some of the available data analysis and post-processing options available today. Note: accurate collection and interpretation of data is the responsibility of the researcher.

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.

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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: Why Study PV Loops?

"Physiologists, and in particular physician physiologists, have often fallen into the trap of measuring certain cardiovascular parameters to explain cardiac performance because they could be measured, rather than because they should be measured."

William J. Mazzei, M.D. 1998

Scientists have historically relied on systemic blood pressure, blood flow, and ventricular pressure to report changes in heart performance. These are all important parameters, but only form part of the picture of heart performance. Pressure-Volume (PV) loops provide a range of hemodynamic parameters which are not readily measurable by other methods; including changes in contractility, elastance, power, energetics and efficiency. What is even more powerful about PV loops is that they provide quantitative measurements of parameters, not just qualitative results. This makes PV loops the single most comprehensive measurement of hemodynamics and cardiac function available.

There are three main areas of cardiovascular assessment where PV loops provide the ideal measurement approach:

1. When it is the best method to measure the contractile parameter of interest including ESPVR and EDPVR.
2. When a comprehensive analysis of cardiac function is needed, such as for phenotyping.
3. When the parameter of greatest interest is unknown during drug or genetic studies.

EXAMPLES OF CARDIOVASCULAR PATHOLOGY THAT CAN BE EXAMINED BY PV LOOPS

- Myocardial Infarction
- Dilated Cardiomyopathy
- Diabetic Cardiomyopathy
- Left Ventricular Hypertrophy
- Right Ventricular Hypertrophy
- Restrictive Cardiomyopathy
- Aortic Valve Stenosis
- Mitral Valve Stenosis
- Aortic Regurgitation (Aortic Insufficiency)
- Mitral Regurgitation
- Right Ventricular Function and Pulmonary Hypertension

All About Contractility

The single greatest advantage of PV loops is the ability to determine the contractility of the heart independent of preload and afterload. By an occlusion procedure (typically the inferior vena cava) a series of pressure-volume loops are created which can be analyzed for a multitude of load independent parameters which are unavailable from other hemodynamic measurement techniques such as echocardiography, MRI and cardiac CT.

PV LOOP MEASUREMENTS

VARIABLE	DESCRIPTION
ESP	End-Systolic Pressure
EDP	End-Diastolic Pressure
ESV	End-Systolic Volume
EDV	End-Diastolic Volume
HR	Heart Rate
Max dP/dt	Maximum Derivative of Pressure
Min dP/dt	Minimum Derivative of Pressure
Max dV/dt	Maximum Derivative of Volume
Min dV/dt	Minimum Derivative of Volume
CO	Cardiac Output
EF%	Ejection Fraction
SV	Stroke Volume
SW	Stroke Work
Ea	Arterial Elastance
maxPwr	Maximum Power
plPwr	Preload Adjusted Power
Eff	Efficiency
PE	Potential Energy
PVA	Pressure-Volume Area
ESPVR	End-Systolic PV Relationship
EDPVR	End-Diastolic PV Relationship
PRSW	Preload Recruitable Stroke Work
E(t)	Time-Varying Elastance
Tau	Isovolumic Relaxation Constant

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PV Workbook RPV-1-wb Rev D 2019
Brochure: RPV-2-fly

Introduction: Cardiac Volume Measurement Methods

Determining the ideal volume measurement technique for heart hemodynamic studies

There are a variety of techniques which can be used to determine common hemodynamic parameters in the research setting. Choosing the best method requires careful consideration of both the technology and the experimental protocol. Four well-established cardiac volume measurement techniques (echocardiography, pressure-volume catheterization, computer tomography (CT) and cardiac magnetic resonance (CMR)) are compared below. Advantages of the Pressure-Volume Catheter method include the relatively low start-up and maintenance costs, and the ability to measure both load dependent and load independent parameters, including contractility.

PRESSURE-VOLUME CATHETER TECHNOLOGY...

- does not use radiation
- no need for a special technician
- high temporal resolution
- good data reproducibility
- low maintenance cost
- low initial price for the system
- no need for ECG gating
- very good volume data reproducibility

	ECHOCARDIOGRAPHY (TRANSTHORACIC)	PV CATHETER (ADMITTANCE)	CARDIAC COMPUTER TOMOGRAPHY (CT)	CARDIAC MAGNETIC RESONANCE (CMR)
REQUIRED HARDWARE	Echo unit & probes	PV unit & catheters	Sectional X-ray for computer reconstruction	Common CMR magnets (6.3-7 Tesla)
SPECIALIZED TECHNICIAN	Often, but not always	No	Yes in most US states	Yes in most US states
PORTABILITY	Unit on wheels	Light & portable	Heavy & stationary	Not portable
MEASUREMENT TECHNOLOGY	Sound waves	Admittance via tetra-polar catheter	Radiation transmission through tissue	Magnetic properties of tissue
USE OF RADIATION	No	No	Ionizing radiation	No
SPECIFICALLY DESIGNED CRADLES FOR ANIMALS	No	No	Yes	Yes
EXAMINATION TIME	5 - 10 min	30 min	20 - 30 min	1 - 3 hrs
USE IN PUBLICATIONS	Very common	Increasingly common	Common	Moderately common
SPATIAL RESOLUTION (AXIAL, LATERAL)	50 μm^2 (2D echo)	Not applicable	100 μm^3 (3D microCT)	200-300 μm^2 (in plane); 1 mm thick
TEMPORAL RESOLUTION	Low	Very high, captures live transient events	Low	High
CONTRAST RESOLUTION	Limited	Not applicable	High	Very high
SIGNAL TO NOISE RATIO	Acceptable	Acceptable	High	Acceptable
COMMON ARTIFACTS (STREAKS & BLUR)	Caused by breathing (need synchronization) can be limited by cardiac gating	Caused by breathing, can be controlled by a ventilator	Caused by breathing (need synchronization), can be limited by cardiac gating	Caused by breathing (need synchronization), can be limited by cardiac gating
MODEL-BASED ESTIMATION (GEOMETRIC)	Volumetry relies on geometric assumptions	PV system corrects geometric assumptions live	Volumetry relies on geometric assumptions	Volumetry relies on geometric assumptions

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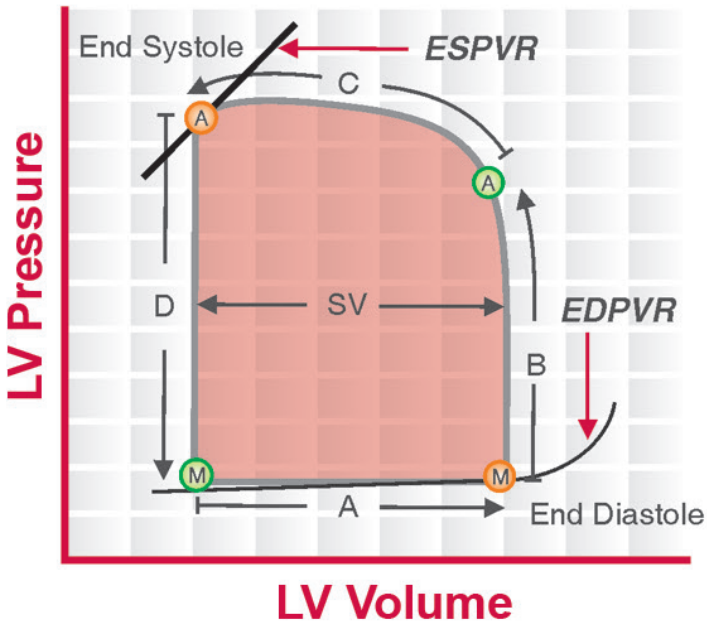
Cardiac Volume Measurement Methods Cont.

	ECHOCARDIOGRAPHY (TRANSTHORACIC)	PV CATHETER (ADMITTANCE)	CARDIAC COMPUTER TOMOGRAPHY (CT)	CARDIAC MAGNETIC RESONANCE (CMR)
REPRODUCIBILITY OF VOLUME DATA	Good	Good	Poor	Poor
REAL TIME/ POST PROCESSING	Volume calculations are based on geometric formulas. Need post-processing	Volume calculations are done in Real-Time. DO NOT need post processing	Volume calculations are based on geometric formulas. Need post processing	Volume calculations are based on geometric formulas. Need post processing
ECG GATING	Reconstruction of images based on gating	Not necessary to use cardiac gating	Reconstruction of images based on gating	Limited by cardiac ECG triggering, respiratory gating
RESPIRATORY GATING	Compulsory	Optional	Compulsory	Compulsory
INTER-USER VARIABILITY	High	Very Low	High	High
VERSATILITY/ OTHER INFORMATION FROM SCAN	Real-time, increases with use of Doppler or 3D echo	Collects multiple parameters (ESPVR, EDPVR, PVA, PRSW, Et, Ea, dp/dt min/ max)	Quantitative measure of tissue density can be used to examine myocardial viability. Ability to produce 3D	High tissue contrast, No contrast agent necessary, ability to acquire 3D
ACCURACY OF VOLUME ESTIMATE	Usually over estimates EDV based on histomorphometry	Usually good EDV estimation based on histomorphometry	Usually over estimates EDV based on histomorphometry	Usually over estimates EDV based on histomorphometry
CONTRACTILITY	Basic (EF%, FS%); load dependent	Excellent with IVC occlusion; load independent	Basic (EF%, FS%); load dependent	Basic (EF%, FS%); load dependent
PRELOAD/ AFTERLOAD DETECTION	Difficult (probe positioning)	Excellent with IVC occlusion	Difficult	Difficult
INVASIVENESS	Moderate	High	Moderate	Moderate
LONGITUDINAL STUDIES	Yes	No	Yes	Yes
INITIAL PRICE OF SYSTEM	High	Low	High	High
PRICE FOR EXPERIMENT	Low	Moderate	High (rental & technician fees)	Very High (rental & longer tech time)
MAINTENANCE COSTS	Low	Low	High	Very high
APPLICATION FIELDS	Cardiovascular (cavity size, valve function etc.), Fluid around heart (pericardial effusion) Cancer (chest tumor biology etc.), Developmental biology (CV morphogenesis), Gene therapy (cardiac sonoporation).	Cardiovascular (left and right cavitary pressure-volume, resistance in the lungs, cardiovascular pressure-volume coupling, cardiac elastance) Isolated working heart (left ventricle pressure-volume).	Cardiovascular (cavity size, valve function, coronary artery calcification etc.), Cancer (chest tumor biology), Developmental biology (CV morphogenesis).	Cardiovascular (cavity size, valve function etc.), Fluid around heart (pericardial effusion) Cancer (chest tumor biology etc.), Developmental biology (CV morphogenesis).

Basics of Pressure-Volume Technology



A. Diastolic Filling
M Mitral Valve Closes
B. Isovolumic Contraction
A Aortic Valve Opens
C. Ejection
A Aortic Valve Closes
D. Isovolumic Relaxation
M Mitral Valve Opens

Simplified sketch of left ventricle pressure volume loop

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Glossary of Pressure-Volume Terms

AFTERLOAD

Afterload is the mean tension produced by a chamber of the heart in order to contract. It can also be considered as the 'load' that the heart must eject blood against. Afterload is therefore a consequence of aortic large vessel compliance, wave reflection and small vessel resistance (LV afterload) or similar pulmonary artery parameters (RV afterload).

ARTERIAL ELASTANCE (Ea)

This is a measure of arterial load and its impact on the ventricle. Calculated as the simple ratio of ventricular end-systolic pressure to stroke volume.

CARDIAC CONTRACTILITY

The intrinsic ability of the heart to contract independent of preload and afterload. On a cellular level it can be characterized as the change of developed tension at given resting fiber length. Used interchangeably with Cardiac Inotropy.

CARDIAC INOTROPY

The ability of the heart muscle to generate force through contraction. Used interchangeably with Cardiac Contractility.

CARDIAC OUTPUT (CO)

Cardiac output is defined as the amount of blood pumped by the ventricle in unit time.

COUPLING RATIO

Indication of transfer of power from the ventricle to the peripheral vasculature.

DERIVATIVE OF PRESSURE (dP/dt)

Reported as max and min rate of pressure change in the ventricle. dP/dt are dependent on load and heart rate. LV dP/dt max occurs before aortic valve closure.

DERIVATIVE OF VOLUME (dV/dt)

Rate of volume change in the ventricle. Maximum and minimum values of dV/dt are normally reported.

EJECTION FRACTION (EF%)

Ejection fraction is the ratio of the volume of blood ejected from the ventricle per beat (stroke volume) to the volume of blood in that ventricle at the end of diastole. It is widely clinically misunderstood as an index of contractility, but it is a load dependent parameter. Healthy ventricles typically have ejection fractions greater than 55%.

E-MAX

Maximum point in the pressure-volume relationship occurring at the end of systole. E-max is directly related to the contractile state of the ventricle chamber. This number is different for each individual heart beat, representing the maximal systolic elastance (E-max) at that moment in time.

END-DIASTOLIC PRESSURE (EDP)

Pressure in the ventricle at the end of diastole.

END-DIASTOLIC PRESSURE VOLUME RELATIONSHIP (EDPVR)

The EDPVR describes the passive filling curve for the ventricle and thus the passive properties of the myocardium. The slope of the EDPVR at any point along this curve is the reciprocal of ventricular compliance (or ventricular stiffness).

END-DIASTOLIC VOLUME (EDV)

Volume in the ventricle at the end of diastole.

END SYSTOLIC ELASTANCE (Ees)

Slope of the end systolic pressure volume relationship.

END-SYSTOLIC PRESSURE (ESP)

Pressure in the ventricle at the end of systole.

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Glossary of Pressure-Volume Terms Cont.

END-SYSTOLIC PRESSURE VOLUME RELATIONSHIP (ESPVR)

The ESPVR describes the maximal pressure that can be developed by the ventricle at any given cardiac chamber volume. This implies that the PV loop cannot cross over the line defining ESPVR for any given contractile state.

END-SYSTOLIC VOLUME (ESV)

Volume in the ventricle at the end of systole.

E(T)

Simplified concept where heart is seen as a purely elastic chamber and the elastance is allowed to vary over time during the cardiac cycle. This cardiac property (including its curve shape and magnitude) can serve as simplified comparison between individual hearts. Relationship is load dependent.

EXCITATION-CONTRACTION COUPLING

The cellular relationship between electrical stimulus and contraction which is primarily influenced by Na^+, K^+ and Ca^{2+} ions and the neural, hormonal and exogenous agents which influence their behavior in the cell.

FRANK-STARLING CURVE

"The heart will pump what it receives"-Starling's law of the heart

SV vs EDP: Afterload dependent measure of inotropy where an increase in inotropy shifts the curve up and to the left; a decrease in inotropy shifts the curve down and to the right.

HEART RATE (HR)

Number of times the heart beats per minute.

ISOVOLUMIC RELAXATION CONSTANT (TAU)

Tau represents the exponential decay of the ventricular pressure during isovolumic relaxation. Several studies have shown that Tau is a preload independent measure of isovolumic relaxation.

LUSITROPY

The relaxation properties of the heart during the diastolic phase.

MYOCARDIAL OXYGEN CONSUMPTION (MVO_2)

Amount of oxygen consumed by the heart as a measure of energy consumption. MVO_2 is dependently correlated with cardiac total mechanical energy (TME).

POTENTIAL ENERGY (PE)

Elastic potential energy of the heart is defined by the area between the ESPVR and EDPVR curves to the left of the PV loop. PE = ESP(ESV-V0)/2 -EDP(EDV-V0)/4 where V0 is the theoretical volume when no pressure is generated.

PRELOAD

Preload is described as the stretching of a single cardiac myocyte immediately prior to contraction and is, therefore, related to the sarcomere length. Since sarcomere length cannot be determined in the intact heart, other indices of preload such as ventricular end diastolic volume or pressure are used.

PRELOAD RECRUITABLE STROKE WORK (PRSW)

PRSW is determined by the linear regression of stroke work with the end diastolic volume. The slope of the PRSW relationship is a highly linear index of myocardial contractility that is insensitive to preload and afterload.

PRESSURE-VOLUME AREA (PVA)

The PVA represents the total mechanical energy (TME) generated by ventricular contraction. This is equal to the sum of the stroke work (SW), encompassed within the PV loop, and the elastic potential energy (PE).

PRESSURE-VOLUME LOOP (PV LOOP)

Graph of pressure (y-axis) and volume (x-axis) of a ventricle over a single cardiac cycle. Several loops are often shown superimposed upon one another.

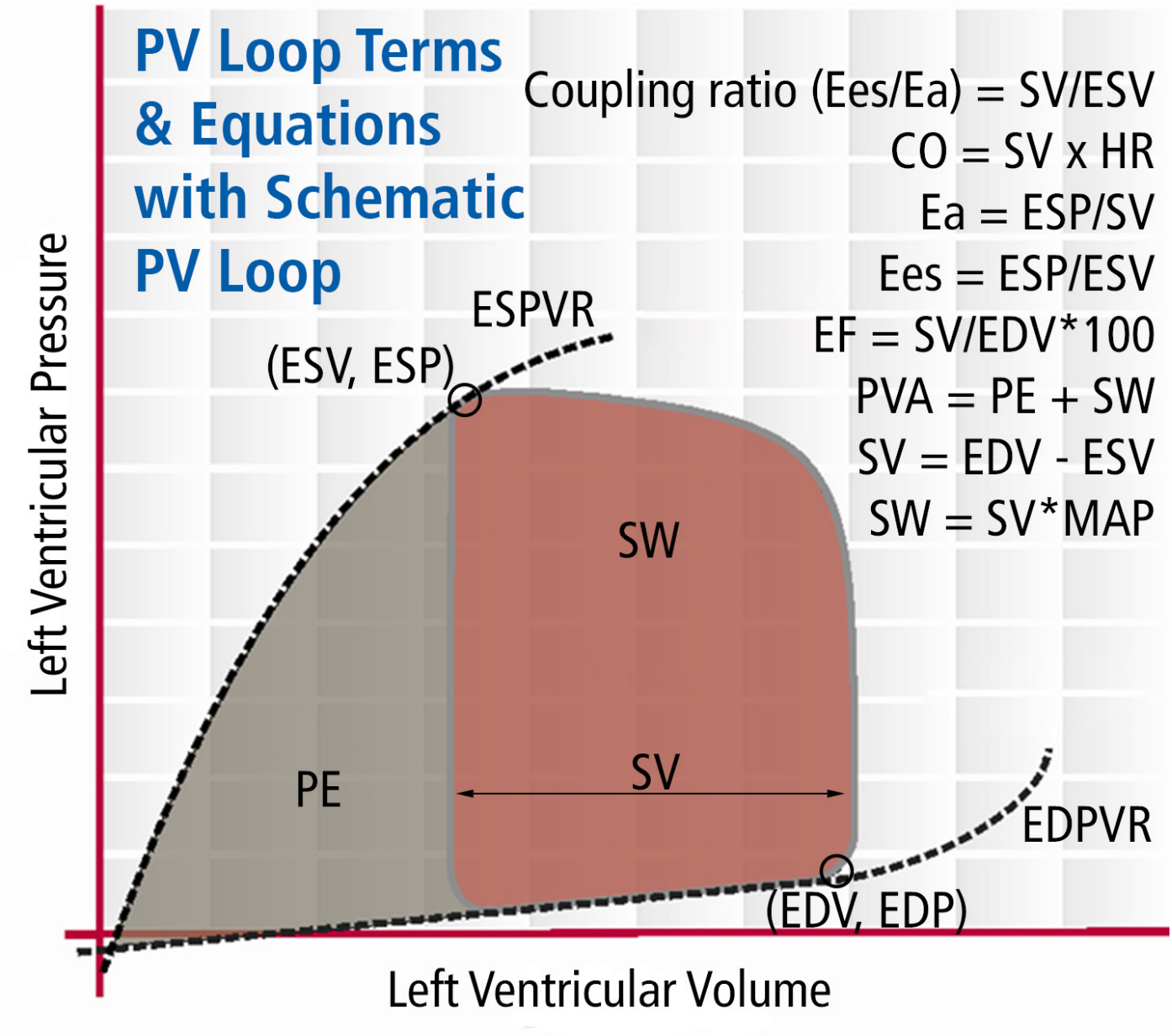
Glossary of Pressure-Volume Terms Cont.

STROKE VOLUME (SV)

Stroke volume is the volume of blood ejected by a ventricle in a single contraction. It is the difference between the end diastolic volume (EDV) and the end systolic volume (ESV).

STROKE WORK (SW)

Ventricular stroke work is defined as the work performed by the left or right ventricle to eject the stroke volume into the aorta or pulmonary artery, respectively. The area enclosed by the PV loop is an estimation of the ventricular stroke work.



Pressure-Volume Conductance Theory of Operation

Deriving ventricular volume from a Conductance Catheter is based on a very simple electrical principle: Ohm's Law:

Voltage (V) = Current (I) X Resistance (R) V = IR

Conductance (G) rather than resistance is the parameter of interest. Since conductance is the inverse of resistance, Ohm's Law can be rewritten as:

Voltage = Current/Conductance V = I/G

Conductance Catheters are comprised of both excitation electrodes and recording electrodes. The excitation electrodes (most distal and proximal electrodes on the Catheter) generate an electrical field inside the heart from the aortic valve to the apex. This field is generated as a result of an alternating current being applied (at a constant magnitude) between these 2 outermost electrodes. The inner recording electrodes measure voltage change which is proportional to a change in resistance.

The electrical field cannot be restricted to just the blood volume and must pass through some of the cardiac muscle. This means that the measured conductance value (G_x) is actually a combination of blood conductance (G_b) and muscle or parallel conductance (G_p).

In 1981, Dr. Baan *et. al.* proposed a relationship between time-varying measurements of total conductance (G_x) to time-varying changes in ventricular volume (Vol) (1). This volume formula takes into account the distance between the recording electrodes (L), blood resistivity (ρ), and the parallel conductance (G_p). It also takes into account the non-uniform nature of the electrical field with the field correction factor, alpha (α).

Alpha is considered an estimate of the slope between conductance derived volume and true volume. Dr. Baan assumed alpha to be 1, as he used a stacked cylinder model for LV. Later he reassessed the LV model changing from a stacked cylinder into a spheroid and used 0.69 for alpha (2).

BAAN'S EQUATION

$$Vol = \frac{1}{\alpha}\rho L^2(G_x - G_p)$$

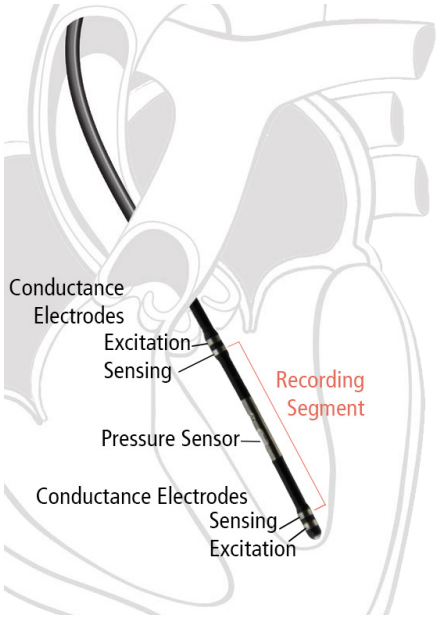
ρ = Blood resistivity

L = Measuring electrode distance

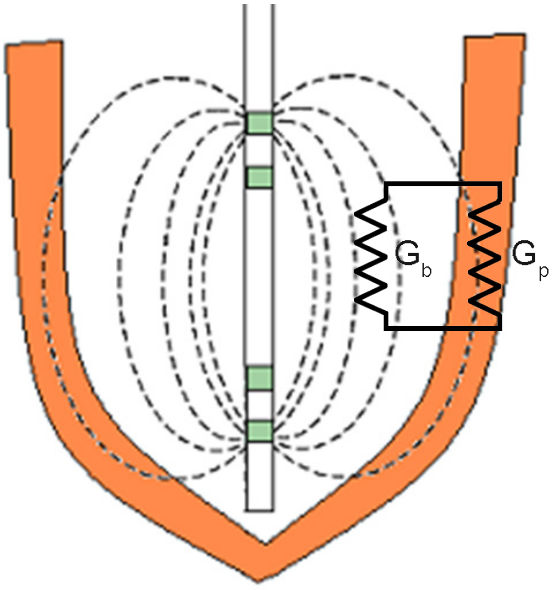
α = Baan's SV correction factor = $(\frac{SV_{conductance}}{SV_{reference}})$

G_x = Measured total conductance

G_p = Baan's parallel/muscle conductance (assumed to be negated by hypertonic saline injection)



Conductance excitation electrodes create an electric field while sensing electrodes measure the voltage change, which allows for the calculation of resistance and conductance.



Conductance uses a circuit model where both blood (G_b) and cardiac muscle (G_m) are conductive and measured together as a single conductance value (G_x) and phase components are ignored.

Pressure-Volume Conductance Theory of Operation Cont.

Recently published alpha values have ranged from 0.5 to 1.01, where the alpha of 0.5 is reported in larger mammals and alpha 1.01 comes from rodent PV research studies (3). Moreover, if the PV Catheter is positioned off-center in the LV, reported alpha ranges from 0.07 to 0.37 in mice (3). For more in depth discussion about alpha, please see article by Steendijk *et. al.* (4).

This approach assumes alpha to be a constant with a single value for a uniform current field distribution (in reality electrical field strength decreases non-linearly with distance). Alpha can be calculated from the SV conductance ratio (see previous page) or by cuvette calibration. Both of these methods give a single constant value for alpha. Parallel or muscle conductance (G_p) is often determined by hypertonic saline injection which temporarily changes blood conductance but not myocardial conductance, allowing for the parallel conductance value to be determined from the graph of changing conductance. This also produces a single constant value for parallel conductance.

REFERENCES

(1) Baan J, et. al. "Continuous stroke volume and cardiac output from intra-ventricular dimensions obtained with impedance catheter." Cardiovasc Res. 1981 Jun;15(6):328-34

(2) Mur G, Baan J. "Computation of the input impedances of a catheter for cardiac volumetry." IEEE Trans Biomed Eng. 1984 Jun;31(6):448–453

(3) Porterfield JE, et. al. "Dynamic correction for parallel conductance, GP, and gain factor, alpha, in invasive murine left ventricular volume measurements." J Appl Physiol. 2009 Dec;107(6):1693-703

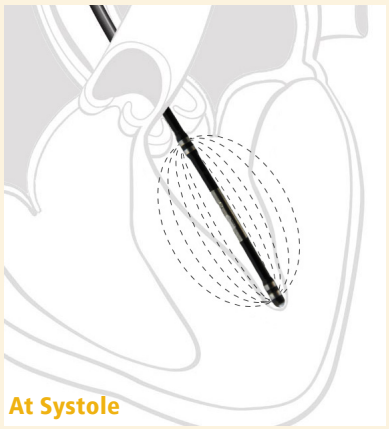
(4) Steendijk P, et. al. "Single and dual excitation of the conductance-volume catheter analysed in a spheroidal mathematical model of the canine left ventricle." Eur Heart J. 1992 Nov;13 Suppl E:28-34

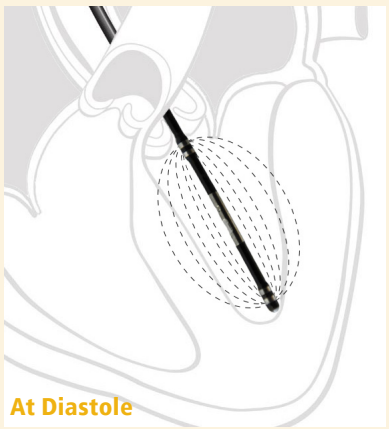
IMPACT OF PHYSIOLOGY ON CONDUCTANCE MEASUREMENTS

At systole there is relatively little blood in the ventricle which means that a larger portion of the electrical field passes through the myocardium. Thus, myocardial resistance contributes more to the total measured conductance than blood at this time. However, because the hypertonic saline bolus method provides an average measurement of muscle contribution, it is typical for the derived volume to be overestimated at systole.

At diastole there is a large quantity of blood in the ventricle and the heart walls have expanded. This means that most of the electrical field is passing though blood with a very small contribution from the myocardium. Thus, the measured conductance value is almost entirely blood conductance. However, the same value of parallel conductance is still subtracted from the total conductance which leads to an under estimation of blood volume.

The electrical field strength decreases in a non-linear manner with increasing field size. This means measurements of blood conductance further from the Catheter do not have the same strength as those nearer to the Catheter. Without correction this leads to an under estimation of total volume. The larger the volume which is being measured, the greater the under estimation. Volume measurements at diastole are thus more prone to under estimation than those at systole. Alpha attempts to correct some of this error but fails to address the non-linearity of the electric field or the varying strength the under estimation has at different phases of the heart cycle.


At Systole


At Diastole

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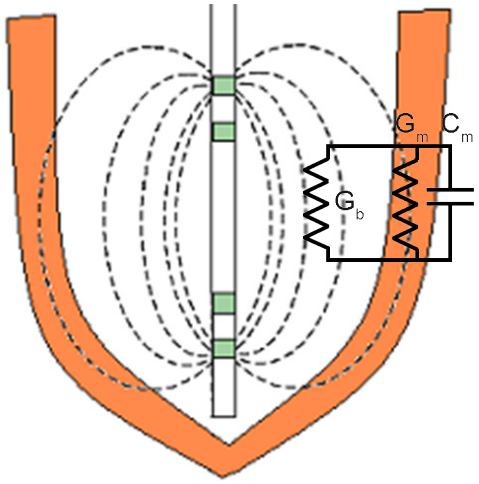
Pressure-Volume Admittance Theory of Operation

Admittance technique is an extension of the Conductance method which measures both resistive and capacitive properties of blood and muscle. In the electric field, blood is purely resistive, but muscle has both capacitive and resistive properties. This allows for separation of the muscle component of conductance from that of blood, using electric field theory.

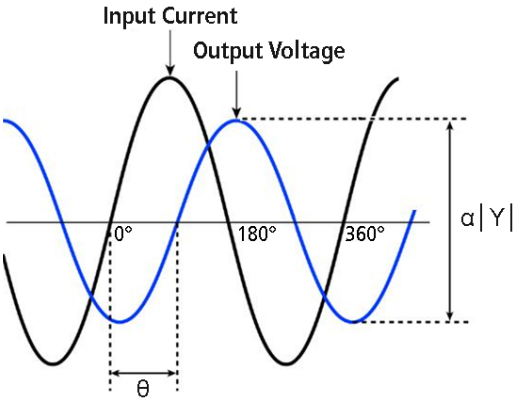
The capacitive property of muscle causes a time (phase) delay in measured signal (see graph at bottom right). By tracking this delay, known as the phase angle, in real time and mathematically relating it to the resistance of the myocardial tissue, the ADV500 System allows continuous, non-invasive tracking of muscle/parallel conductance (G_m) throughout the heartbeat (1). The phase angle reports heart tissue intrusion into the field as the heart contracts and expands, and as expected, this measurement is greatest at systole and lowest at diastole. This provides a great advantage over classical conductance volumetry which treats parallel conductance as a constant, rather than a dynamic variable which changes throughout the cardiac cycle.

The ADV500 system employs an equation developed by Dr. Chia-Ling Wei to convert conductance to volume instead of the traditional Baan's equation. In Baan's equation the Field Correction Factor, alpha (α), is assumed to be constant despite the non-linear nature of the electrical field (2). However, Wei's equation corrects for the nonhomogeneous nature of the Catheter's electrical field distribution by assuming a non-linear relationship between conductance and volume, gamma (γ), thus improving accuracy over a wider volume range.

To measure blood volume in real time values are needed for myocardial conductivity and permittivity (for σ/ε ratio/heart type), blood resistivity (ρ), and reference stroke volume (SV). Default values for 'Heart Type' (S/E ratio) are provided and most commonly used (3). However, researchers can study this value using a tetrapolar surface probe provided with the ADV500. Stroke volume can be measured via other technologies.



Admittance uses a circuit model where blood is conductive (G_b) and cardiac muscle is both conductive (G_m) and capacitive (C_m).



The output voltage shows a "delay" compared to the input voltage signal used to generate the electric field. The signal delay, caused by myocardial capacitance, is measured in terms of degrees and is referred to as "Phase angle θ." The admittance magnitude (conductance) is impacted by both the blood and muscle.

WEI'S EQUATIONS

$$Vol = \frac{1}{(1-\frac{G_b}{\gamma})}\rho L^2(G_b)$$

ρ = Blood resistivity

L = Measuring electrode distance

G_b = Measured blood conductance

SV = Stroke volume

Field Correction Factor:

$$\gamma = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

$$a = SV - \rho L^2(G_{b-ED} - G_{b-ES})$$

$$b = -SV \cdot (G_{b-ED} + G_{b-ES})$$

$$c = SV \cdot G_{b-ED} \cdot G_{b-ES}$$

REFERENCES

(1) Wei CL, et. al. "Evidence of time-varying myocardial contribution by in vivo magnitude and phase measurement in mice." Conf Proc IEEE Eng Med Biol Soc 2004. 5:3674-7.

(2) Wei CL, et. al. "Nonlinear conductance-volume relationship for murine conductance catheter measurement system." IEEE Trans Biomed Eng 2005. 52:1654-61.

(3) Raghavan K, et. al. "Electrical conductivity and permittivity of murine myocardium." IEEE Trans Biomed Eng. 2009. 56:2044-53.

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Comparing Conductance vs Admittance

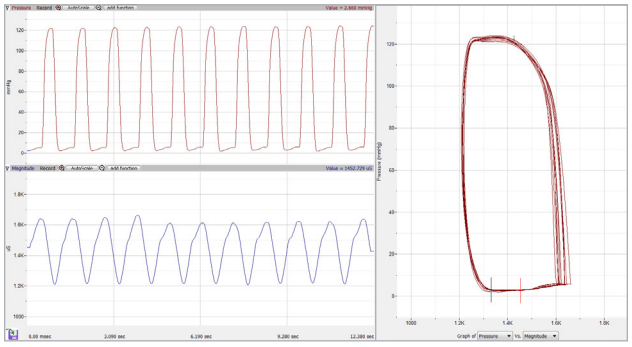
The Scisense ADV500 Pressure-Volume System is capable of being used in either Conductance or Admittance mode. Both methods have value depending on what the researcher is looking to observe.

Conductance

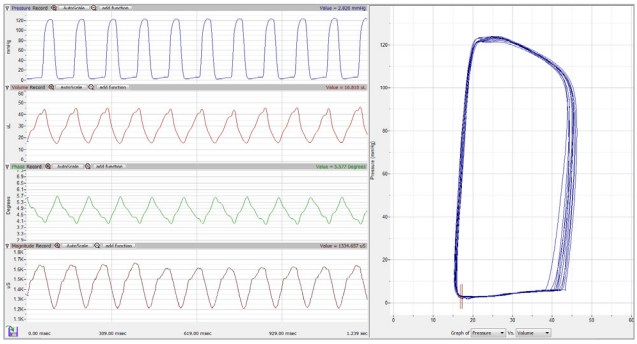
- Measures voltage magnitude
  - Harder to determine position of Catheter in ventricle
- Uses Baan's equation to determine volume
- Parallel conductance (G_p) is assumed constant
  - Parallel conductance determined from hypertonic saline injection after the experiment
- Field Correction Factor is assumed constant (alpha)
  - Requires empirical reference stroke volume to derive α or an approximation (typically 1) can be used
- Volume calculation is done post experiment with no chance to correct for protocol or surgical errors.
- Tends to overestimate volume due to constant nature of α as observed with echocardiography.
- Traditional technique with a solid body of papers that validate the basic principle of conductance catheter volumetry.

Admittance

- Measures voltage magnitude and phase angle
  - Phase angle useful in locating Catheter in ventricle
- Uses Wei's equation to determine volume
- Muscle conductance (G_m) varies throughout cardiac cycle
  - Parallel conductance determined from phase shift in real-time (no hypertonic saline injection required)
  - Requires sigma/epsilon ratio (conductivity/ permittivity) of heart muscle. Default values are commonly used, or can be measured using tetrapolar Calibration Probe.
- Field Correction Factor is non-linear (gamma)
  - Requires empirical reference stroke volume to derive gamma (γ)
- Volume calculation is in real time. Corrections to experimental protocol or surgery can be made before experiment is concluded.
- Closer approximation to absolute systolic and diastolic volume as observed with echocardiography.
- Innovative technology that builds directly on the foundation of conductance catheter volumetry.



Conductance method measures pressure and magnitude in real-time, creating pressure-magnitude loops. Volume can only be calculated post-experiment.



Admittance method measures pressure, volume, phase and magnitude in real-time, creating pressure-volume loops.

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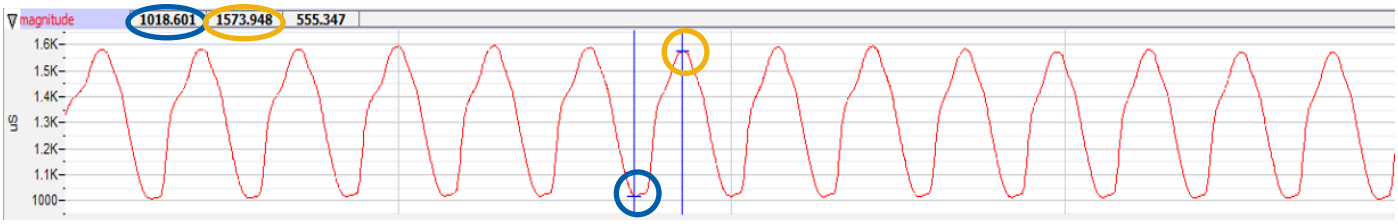
Understanding Magnitude & Phase Signals

Magnitude (Conductance) Signal

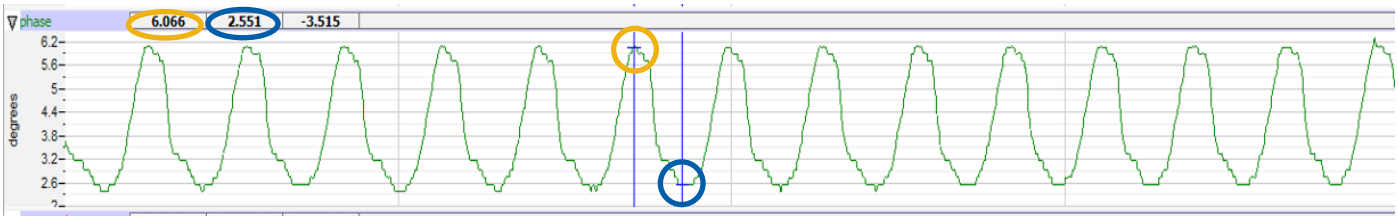
- Sinusoidal pattern of the wave
- Concentrate on Magnitude amplitude and range at the same time
- Values dependant on animal species, see typical values in table below



Example of good magnitude signal in a mouse. Minimum values are circled in blue and maximum values are circled in orange. Note that the magnitude (1019 - 1574 µS) falls within the expected range (900 - 2000 µS) and that the amplitude (555 µS) is also close to expected range.

Phase (Admittance) Signal

- Sinusoidal pattern of the wave
- Concentrate on Phase amplitude and range at the same time
- Values dependant on animal species, see typical values in table below



Example of good phase signal in a mouse. Minimum values are circled in blue and maximum values are circled in orange. Note that the phase angle (2.5 - 6.1°) falls within the expected range (2 - 8°) and that the amplitude (3.5°) is also close to expected range.

ANIMAL	PHASE ANGLE	PHASE AMPLITUDE	MAGNITUDE RANGE	MAGNITUDE AMPLITUDE
Mouse	2 - 8°	2.0°	900 - 2000 µS	200 - 500 µS
Rat	2 - 6°	2.0°	1400 - 2600 µS	600 - 1000 µS
Rabbit	2 - 6°	2.0°	8 - 14 mS	2 - 3 mS
Small Dog	1 - 5°	1.5°	10 - 16 mS	2 - 3 mS
Large Dog (>15 kg)	1 - 5°	1.5°	12 - 18 mS	2 - 4 mS
Small Swine	1 - 3°	1.5°	12 - 18 mS	2.5 - 4 mS
Large Swine (>65 kg)	1 - 3°	1.5°	15 - 30 mS	4 - 6 mS
Sheep	1 - 3°	1.5°	14 - 22 mS	4 - 5 mS
Cow	2 - 5°	2.0°	20 - 40 mS	10 - 13 mS

Typical values should be used as a guidelines only

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Understanding Afterload

Afterload is the mean tension produced by a chamber of the heart in order to contract. It can also be considered as the 'load' against which the heart must eject blood. Afterload is composed of these major parameters: myocardial wall stress, arterial blood pressure, arterial resistance and arterial impedance. A more mathematically precise model of wall stress accounting for afterload comes from a modification of the LaPlace Law which states that wall tension (T) is proportional to the pressure (P) times radius (r) for thin-walled spheres or cylinders. Therefore, wall stress is wall tension divided by wall thickness.

$$\sigma \propto \frac{P \times r}{2h}$$

σ = ventricular wall stress

P = ventricular pressure

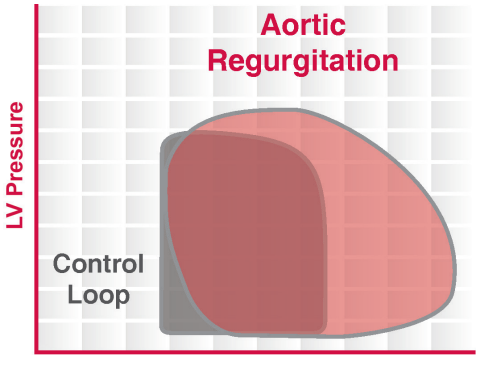
r = ventricular radius

h = wall thickness

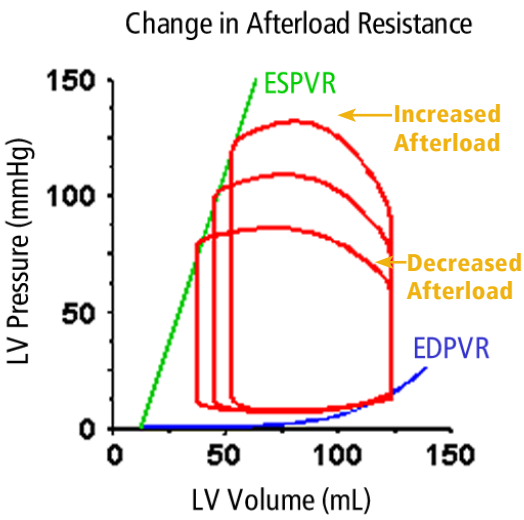
From this relationship it is apparent that wall stress (afterload) increases when the aortic or arterial pressure increases, ventricular radius increases (ventricular dilation), or wall thickness decreases.

Overall, wall stress is contractility dependent and is not constant during the contraction. LV cavity pressure, wall thickness and curvature vary with preload.

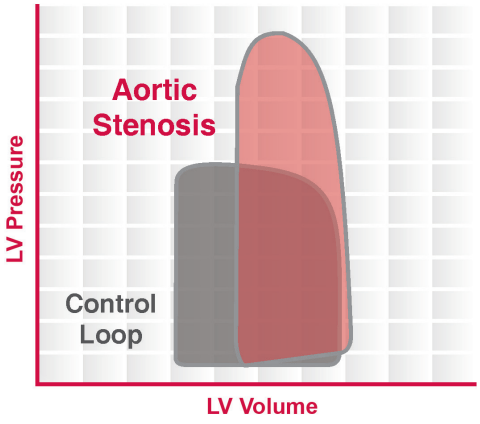
As mammals posses closed cardiovascular circuits, arterial blood pressure, resistance or impedance will have impact on the heart. Left ventricular afterload is affected by various disease conditions. Hypertension increases the afterload since the LV has to work harder to overcome the elevated arterial peripheral resistance and decreased compliance. Aortic valve diseases like aortic stenosis and insufficiency (regurgitation) also increase the afterload whereas mitral valve regurgitation decreases the afterload. Long-term afterload increases can lead to decreased stroke volume and deleterious cardiac remodeling.



Schematic drawing of healthy (control) and disease state (aortic regurgitation) LV PV loops.



Different PV loops are obtained with different preloads, modeled by constant contractility (ESPVR and EDPVR boundaries) and afterload. Image courtesy of Burkhoff D, Boston 2013, Transonic lectures.



Schematic drawing of healthy (control) and disease state (aortic stenosis) LV PV loops.

Understanding Afterload Cont.

Afterload cannot be measured directly, but several methods for assessing afterload indirectly are available. This is normally accomplished by characterizing the interaction between the heart and the arterial system.

ECHOCARDIOGRAPHY

Echocardiography (both transthoracic and transesophageal) gives reliable measures of end systolic surface areas and wall thickness. In combination with ventricular pressure measurement this allows for the calculation of ventricular wall stress as described previously. Additionally, long axis Doppler echocardiography can measure decreases in stroke volume (SV) during the period of increased afterload.

COMBINED PRESSURE AND FLOW

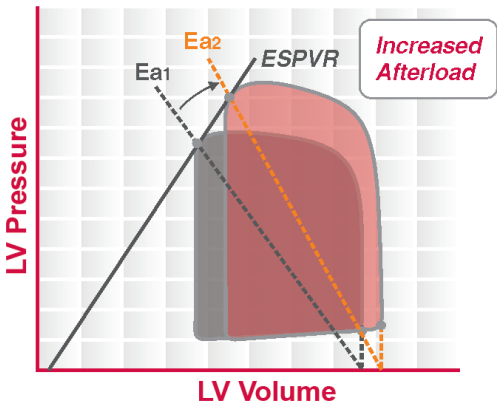
Combination of pressure and flow measurement (transit-time ultrasound Flowprobe and Pressure Catheter) can be used to determine afterload. Using flow as a measurement of cardiac output (CO) and both mean arterial pressure (MAP) and central venous pressure (CVP) can help to determine total peripheral resistance (TPR). Most investigators however omit CVP due to its minimal influence on the total, therefore:

TPR= (MAP /CO) = (MAP /SV*HR)

PRESSURE-VOLUME LOOPS

Pressure-volume loops characterize afterload by total mechanical load on the ventricle during the ejection. During many temporary or chronic (e.g. peripheral vascular hypertension) disease states the PV loops move to the right while both ESP and peak chamber pressure increases and SV decreases. As the ESP increases changes of arterial elastance Ea (the ratio of ventricular chamber end-systolic pressure to stroke volume i.e. Ea=ESP/SV) will takes place along with increase of chambers afterload. To compensate for this peripheral vascular resistance change, necessary adjustment of heart rate (increase) will take place.

On a cellular level, during increased afterload, heart muscle cells have to increase their metabolism while starting to use an oxidative phosphorylation pathway to obtain more ATP to handle calcium homeostasis and, ultimately, to contract. As this increase persists and excitation-contraction mechanisms are stretched to the maximum, it leads to depletion of calcium, and energy homeostasis. Moreover, an increase in oxygen consumption will be characterized by an increase in PVA (pressure volume area) at the beginning of afterload challenge.



Schematic drawing of healthy (control) and disease state (increased afterload) LV PV loops.

REFERENCES

(1) Franklin RC, et. al. "Normal values for noninvasive estimation of left ventricular contractile state and afterload in children." Am J Cardiol. 1990 Feb 15; 65(7): 505-10.

(2) Veras-Silva AS, et. al. "Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats." Am J Physiol. 1997 Dec; 273: H2627-H2631

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Understanding Preload

Preload is known as the load imposed on the ventricle at the end of diastole. Preload and its relationship to wall stress can be described by LaPlace's Law as pressure at the end diastole times radius of chamber at the end diastole divided by 2 times thickness of the chamber wall thickness at the end diastole.

$$\sigma \propto \frac{P \times r}{2h}$$

σ = ventricular wall stress

P = ventricular pressure

r = ventricular radius

h = wall thickness

On a cellular level, preload is defined as the maximum degree of myocardial fiber stretch or tension (stress) before ventricular contraction, determined by the mean sarcomere length at the end of diastole. Since sarcomere length cannot be determined in the intact heart, other indices of preload such as ventricular end diastolic volume (EDV) or pressure (EDP) are used. In general, EDV offers a better estimation of preload than EDP.

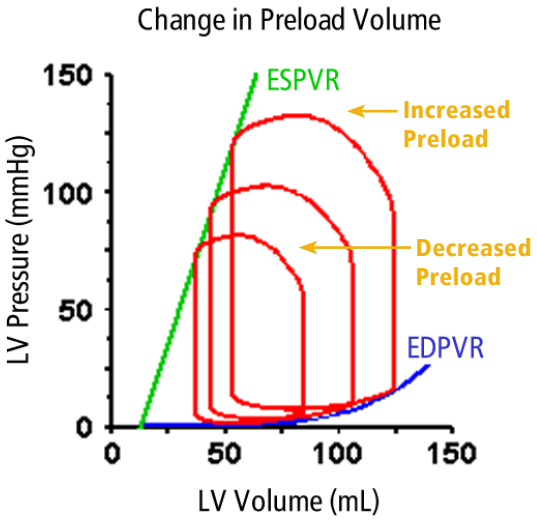
The relationship between the changes in preload and stroke volume depends on the morphology and Frank-Starling curve, which are determined by the contractile capacity of the heart and the ventricular afterload. Increasing preload increases stroke volume by a non-linear relationship. Cardiac preload can also be described as the passive filling properties of ventricles.

FACTORS CAUSING PRELOAD (EDV) INCREASE

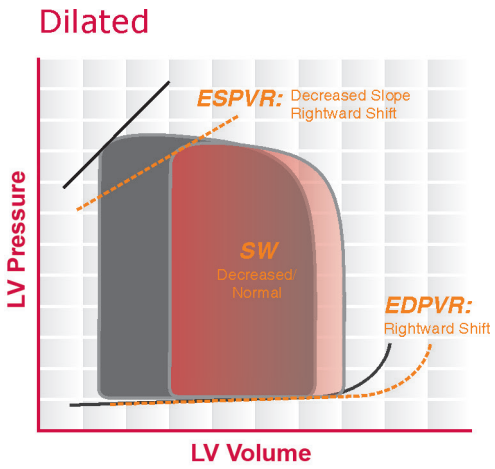
- Increased ventricular compliance
- Venoconstriction
- Increased venous return (skeletal muscle activity and respiratory activity during physical activity or position and gravity)
- Decreased heart rate (increased filling time)
- Neuro-endocrine stimulation of venous tone
- Increased blood volume (e.g. post-transfusion)

FACTORS CAUSING PRELOAD (EDV) DECREASE

- Compliance of ventricle decreases or stiffness increases (multiple muscle diseases leading to hypertrophy, post-tissue graft implantation, etc.)
- Increased heart rate (reduced filling time)
- Venodilation (peripheral or central) causing blood to pool in legs, abdomen, liver etc.
- Atrial arrhythmias (impaired contraction)



Different PV loops are obtained with different preloads, modeled by constant contractility (ESPVR and EDPVR boundaries) and afterload. Image courtesy of Burkhoff D, Boston 2013, Transonic lectures.



Schematic drawing. During dilated cardiomyopathy, due to damage of the myocardium chambers, remodeling increases both ED and ES volumes (PV loop moves to the right). Frank-Starling curves shifts down and to the right due to a decrease in contractility. During this dysfunction, stroke volume is also reduced and preload increases as a consequence. LV EDP is near normal at low volumes but becomes elevated at high diastolic volumes as the myocardium is rendered non-compliant by hypertrophy, fibrosis or ischemia.

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Understanding Preload Cont.

Preload can be measured by a variety of methods.

THERMODILUTION

Thermodilution can be used to obtain a global EDV index to evaluate biventricular preload. Alternatively, right ventricle EDV can be obtained using a pulmonary artery catheter with a rapid response thermistor in the right ventricle.

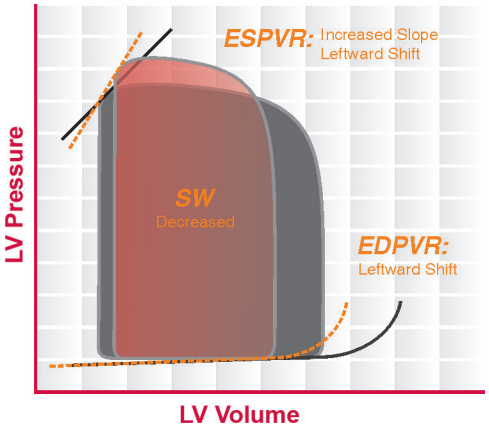
ECHOCARDIOGRAPHY

Echocardiography (both transthoracic and transesophageal) gives reliable measures of end diastolic surface areas. End-diastolic volume can then be calculated using Simpson's method or a similar approach. Long axis Doppler echocardiography can measure increases in stroke volume (SV) during the period of increased preload.

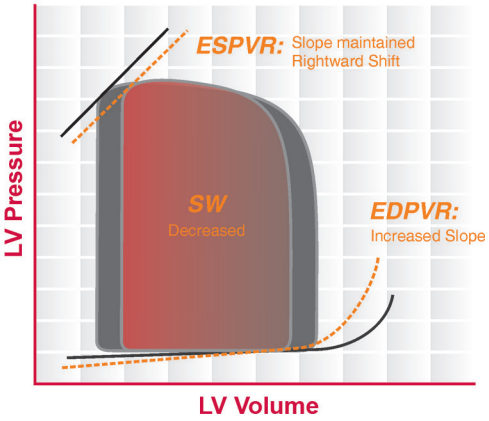
PRESSURE-VOLUME LOOPS

Pressure-volume loops give a direct measurement of end diastolic volume as derived by admittance technology. At the same time, PV loops have the advantage of also providing information on heart contractility (load independent) based on the end systolic and end diastolic pressure volume relationships (ESPVR and EDPVR).

Hypertrophic



Restrictive



Schematic drawings of both left ventricular hypertrophy and restrictive cardiomyopathy exhibit a reduced end diastolic volume (EDV). As the LV becomes more stiff and less or non-compliant, PV loop leftward shift occurs.

In hypertrophic cardiomyopathy, further myocardial wall remodeling leads to concentric hypertrophy and heart failure. Decreasing of all load parameters (e.g. EDV, SV and EF) occurs as compliance of the ventricle further decreases and stiffness of the chamber increases. When preload rapidly decreases, chamber pressure increases leading to steeper ESPVR.

During restrictive cardiomyopathy, stiffening of the myocardium occurs due to myocarditis leading to reduction of all load based parameters (e.g. EDV, SV, EF). While ESPVR might be unchanged at the beginning, diastolic dysfunction becomes obvious as characterized by an EDPVR shift indicative of heart failure.

Understanding Contractility: Cardiac Inotropy

Cardiac contractility is the intrinsic ability of heart muscle to generate force and to shorten, ideally independent of changes in heart rate (HR), preload or afterload. In that respect, cardiac chamber pressure-volume measurement is the most reliable index for assessing myocardial contractility in the intact circulation, being almost unaffected by changes in preload and afterload.

Contractility is regulated by many mechanisms:

- The parasympathetic and sympathetic nervous systems through catecholamines (circulating, delivered) control contractile force and ensure the coupling between heart performance and peripheral circulation. Catecholamines increase contractile force by the β adrenoceptor-adenylyl cyclase system or by stimulation of α-receptors. Through protein phosphorylation of L-type calcium channels, increase of calcium influx and activation of ryanodine receptors (RyRs) occur to further increase the sarcoplasmatic reticulum calcium release. At the same time, other processes speed up calcium accumulation in the sarcoplasmatic reticulum to allow faster cardiomyocyte relaxation. Parasympathetic action (vagus nerve) has a beneficial effect on cardiac contractility by improving hemodynamics, including decreasing HR and pressure. Vagus nerve stimulation also effectively suppresses arrhythmias, including premature ventricular contractions (2).
- Stroke volume is critically dependent on inotropy. When sarcomere length increases or during preload augmentation, contractile force and stroke volume increases correspondingly, based on the Frank-Starling mechanism.
- Myocardial force development is HR dependent (Bowditch effect). In healthy myocardium the effect is expressed as an increase of heart rate by cardiac pacing that is able to a produce progressive increase in the force of contraction for a few beats (isometric force development) and then remains at a higher plateau (Positive Staircase effect). Functionally, with increasing HR, more calcium enters the cardiomyocyte and is accumulated into the sarcoplasmatic reticulum, while becoming accessible for release in the next contraction, resulting in increased contractile force. The inverse effect occurs when HR is decreased (Negative Staircase).
- Increase in afterload causes an increase in ventricular contractility (inotropy) due to the activation of catecholamines. This effect allows the myocardium to compensate for an increased end-systolic volume and decreased stroke volume that occurs when aortic blood pressure increases. It is called the Anrep effect. Without this effect in place, an increase in aortic blood pressure would create a drop in stroke volume that would compromise circulation to peripheral and visceral tissues.

CONTRACTILITY AND HEART FAILURE

MYOCARDIUM	RT (mN/mm²)	PDF (mN/mm²)	WORK (%)
Non-failing	11.2±1.3	14.5±4.4	136±11
Failing	16.3±1.5	12.7±4.5	74±7

Resting tension (RT) and work are significantly different.
Peak developed force (PDF) is not (1).

During heart failure, changes in the gene expression occur (from the adult to fetal pattern) leading to lowering of systolic calcium release and diastolic calcium reuptake. These molecular changes lead to physiological (heart function/hemodynamic) alterations that heavily influence inotropy. Studies in isolated myocardium have shown that increasing contraction rate does not increase contraction force or work in failing myocardium as it does in normal myocardium. However, the Frank-Starling mechanism is still intact in failing myocardium. This does not translate to increased work with increased sarcomere length due to the higher resting tension of failing cardiac muscle. Additionally, failing myocardium has reduced extent of shortening as compared to non-failing myocardium. When cardiac muscle length is increased close to its maximum (maximal stretch) in non-failing myocardium the maximal myocardial work increases with accompanied isometric force development as compared to failing myocardium where the myocardial work is decreasing when cardiac muscle is stretched to its maximum length (1).

Understanding Contractility: Cardiac Inotropy Cont.

Positive Inotropic Agents (Increase Contractility)

TYPE OF AGENT	MECHANISM/ EFFECTS	EXAMPLE AGENT(S)
Calcium	Increases available calcium for binding.	Calcium
Calcium Sensitizer	Increases myocyte calcium sensitivity and binding to cardiac troponin C in a calcium-dependent manner.	Levosimendan
Cardiac Myosin Activators	Targets and activates myocardial ATPase and improves energy utilization. This enhances effective myosin cross-bridge formation and duration.	Omecamtiv
Beta Agonists	Stimulates adenylyl cyclase activity and opening of calcium channels.	Dobutamine, Isoproterenol, Xamoterol
Intrinsic Catecholamines	Increases heart rate, blood pressure and glucose levels.	Dopamine, Epinephrine (adrenaline), Norepinephrine (noradrenaline)
Cardiac Glycosides	Competes with K^+ ions for the same binding site on the Na^+/K^+ ATP-ase pump in cardiomyocytes and decreases its function. This causes an increase in the level of Na^+ in cardiomyocytes, which leads to a rise in the level of intracellular Ca^{2+} because the Na^+/Ca^{2+} exchanger on the plasma membrane depends on a constant inward Na^+ gradient to pump out Ca^{2+}.	Digitalis, Digoxin, Ouabain
Phosphodiesterase-3 Inhibitors	Protects cyclic AMP from its degradation, increases inotropy. Decreases afterload by vasodilatation	Milrinone, Amrinone, Enoximone, Papaverine
Insulin	Exerts Ca^{2+} dependent and independent positive inotropic effects through a phosphatidylinositol-3-kinase (PI3K) dependent pathway.	Insulin
Glucagon	Stimulates the cardiac Ca^{2+} current by activation of adenylyl cyclase and inhibition of phosphodiesterase.	Glucagon

Negative Inotropic Agents (Decrease Contractility)

TYPE OF AGENT	MECHANISM/ EFFECTS	EXAMPLE AGENT(S)
Beta Blockers	Block the action of endogenous catecholamines by interfering with the binding of adrenaline and noradrenaline to their receptors.	Acebutolol, Bisoprolol, Propranolol, Atenolol
Calcium Channel Blockers	Block voltage-gated calcium channels in cardiac muscle.	Verapamil, Diltiazem
Class IA Antiarrhythmic (fast channel blockers)	Block open Na^+ channels, prolonging cardiac action (affecting QRS complex). This results in slowed conduction and ultimately the decreased rate of rise of the action potential.	Quinidine, Procainamid
Class IB Antiarrhythmic	Na^+ channel blockers cause a reduction of the rate of rise of intracellular Na^+.	Lidocaine
Class IC Antiarrhythmic	Na^+ channel blockers	Flecainide, Propafenon
Class III Antiarrhythmic	Have β-like and K^+-like actions, increasing the refractory period via Na^+ and K^+ channel effects, and slowing intracardiac conduction of the cardiac action potential.	Amiodarone

Understanding Contractility: Cardiac Inotropy Cont.

FACTORS AFFECTING THE LEVEL OF INOTROPIC (CONTRACTILE) STATE

Intrinsic

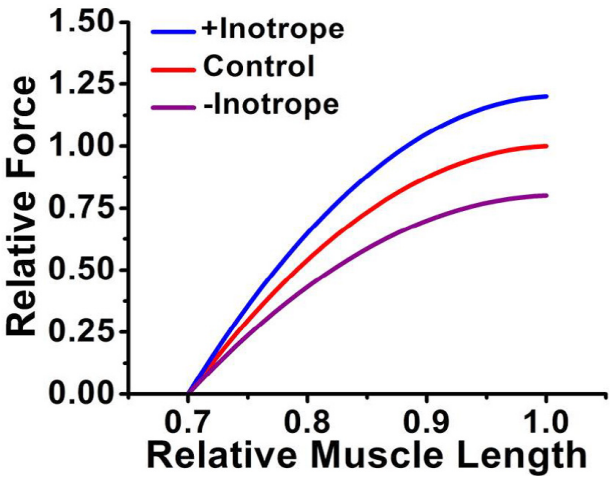
- Affinity of myocardium for calcium changes (insufficient blood flow, ischemia)
- Damage of heart muscle (alteration of numbers of contractile units)
- Calcium release and re-uptake
- Hormones (Glucagon, Insulin)
- Temperature

Extrinsic

- Pharmacological agents (β-agonist, β-blockers, isoflurane)
- Release of norepinephrine into myocardium when postganglionic sympathetic axis is activated
- Release of acetylcholine when parasympathetic axis is activated
- Increase of extracellular calcium concentration

ASSESSMENT OF CELLULAR CONTRACTILITY

- Intact myocardial cells are phenotypically different based on their sarcomere length, as they are localized in different cell sheets of heart. Inside (sub-endocardial) cell layer is stiffer as compared to outside (sub-epicardial) (3).
- Cell sheets localized at sub-endocardium react on pre-load force activation better, as they have longer end-diastolic sarcomere length (4).
- Phenotypic differences might further guide contraction of the whole heart. As during systole, earlier pre-contraction of sub-endocardium might be beneficial for the sub-epicarial, less-stiffer layer (5).
- Importantly, cells from the RV base and apex are narrower as compared to LV at these locations as measured in guinea pigs. This might limit their force-generation, but as they are longer, it might be an advantage to their length-change potential (6).



An increase in inotropy is associated with an increase in the strength of contraction (force) for the same stretch or preload (muscle length). A decrease in inotropy decreases contraction strength (1). Changes in the inotropic state of the myocardium produce changes in performance (force development, extent of shortening) independent of preload and afterload.

Image courtesy of Burkhoff D, Boston 2013, Transonic lectures

REFERENCES

(1) Holubarsch C, et. al. "Shortening versus isometric contractions in isolated human failing and non-failing left ventricular myocardium: dependency of external work and force on muscle length, heart rate and inotropic stimulation." Cardiovasc Res. 1998 Jan;37(1):46-57.

(2) Zheng C, et. al. "Vagal stimulation markedly suppresses arrhythmias in conscious rats with chronic heart failure after myocardial infarction." Conf Proc IEEE Eng Med Biol Soc. 2005; 7:7072–7075.

(3) Cazorla O, et. al. "Length–tension relationships of sub-epicardial and sub-endocardial single ventricular myocytes from rat and ferret hearts." J Mol Cell Cardiol. 2000. 32 (5):735–744.

(4) Bryant SM, et. al. "Regional differences in electrical and mechanical properties of myocytes from guinea-pig hearts with mild left ventricular hypertrophy." Cardiovasc Res. 1997. 35 (2):315–323.

(5) Ashikaga H, et. al. "Transmural myocardial mechanics during isovolumic contraction." JACC Cardiovasc Imag. 2009. 2(2):202–211.

(6) Bollensdorff C, et. al. "Assessment of contractility in intact ventricular cardiomyocytes using the dimensionless 'Frank-Starling Gain' index." Pflugers Arch. 2011 Jul;462(1):39-48.

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Understanding Lusitropy

Lusitropy describes the relaxation properties of the heart during the diastolic phase. Left Ventricle (LV) relaxation begins during late ejection and continues throughout an early rapid filling and ends fully relaxed by diastasis, before the atrial systole begins. Diastolic (lusitropic) properties can be described by both active relaxation and passive diastolic properties.

This active relaxation during diastole is a spatially non-uniform process, based on different rates and amounts of untwisting during periods of isovolumic ventricular relaxation (IVR). Twisting of the myocardial tissue leads to storage of potential energy that is freed in early ventricular diastole during untwisting. As the LV wall is composed of helically woven muscle layers and sheets, including extracellular matrix, all are assembled in interwoven layers such that fiber orientation is modified both transmurally and along the long axis of the ventricle (1). This LV geometric arrangement generates the spatially and temporally unique relaxation pattern accounting for unique, heart specific lusitropic patterns (1). Additionally, since LV and RV share the common septum, direct diastolic ventricular interaction is important to consider lusitropy when assessing the diastolic properties.

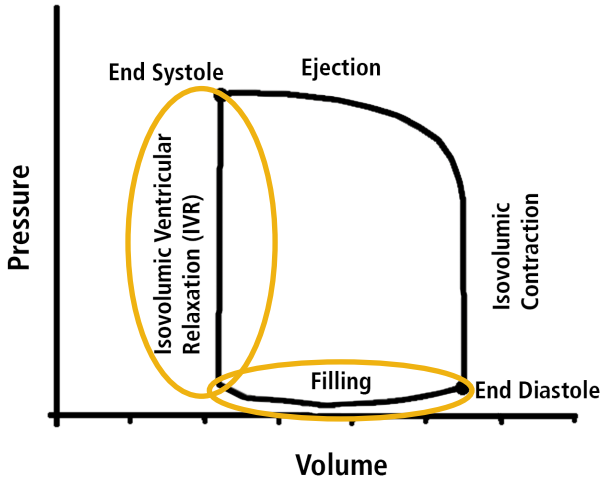

Fig. 1: Simplified sketch of LV PV loop. Diastolic phase includes isovolumic ventricular relaxation (IVR) and filling.

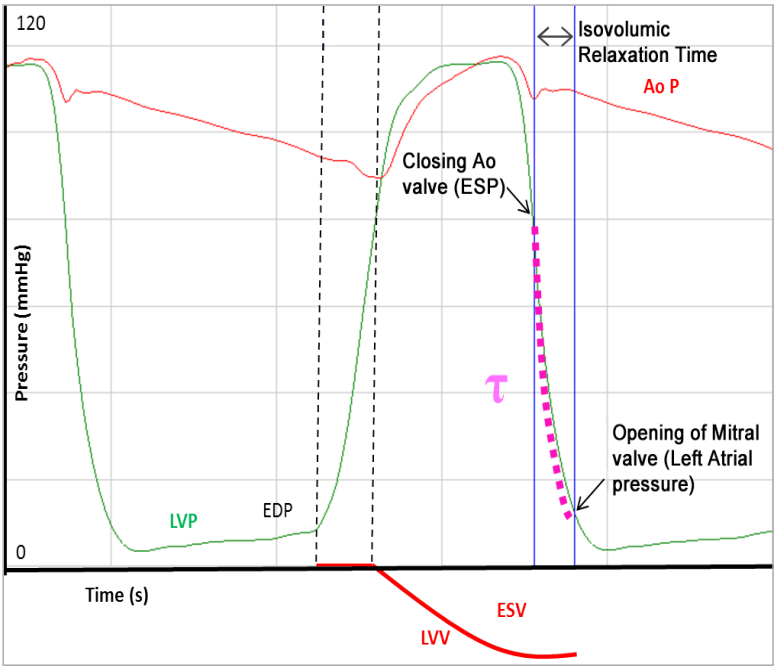

Fig. 2: The decay of LV pressure during the isovolumic ventricular relaxation (IVR) of diastole follows a roughly exponential time course. Active relaxation can be characterized by Tau, the segment of pressure contour between aortic valve closure and the mitral valve opening.

It is important to note that Tau has multiple methods of expression. Tau was originally used by Weiss to describe the IVR of LV (3). Raff and Glantz proposed an alternative method to express Tau, referred to as Tau Glantz (4). The latest IVR Tau logistic was proposed and described in 1995 by Dr. Suga in Japan (5).

Understanding Lusitropy Cont.

ACTIVE RELAXATION PROPERTIES

- Indexed by Tau (isovolumic relaxation time, also known as time of pressure decay) IVR is from aortic valve closure to mitral valve opening
- dP/dt_{min} (is not as precise when compared to Tau, since dP/dt_{min} depends on the peak aortic pressure and timing of aortic valve closure) (2)
- Impacted by heart rate (HR)
- On cellular level, relaxation is energy consuming process requiring ATP as release of calcium from sarcomere requires SERCA (sarco-endoplasmatic reticulum Ca-ATPase) for its re-uptake.

An increase in Tau indicates impairment of active properties of diastolic relaxation. Isovolumic relaxation and Tau are influenced by:

- Left atrial - left ventricle pressure gradient
- LV elastic recoil
- Chamber relaxation
- Mitral orifice area
- Heart rate
- Energy supply (Tau increases during MI and post-ischemia)
- Beta-stimulus (Tau decreases with β-adrenergic stimulation)

During many LV disease states (i.e. LV hypertrophy, LV ischemia, diabetic cardiomyopathy etc.) active relaxation is delayed.

When active relaxation is inadequate in early diastole, LV chamber relaxation might become incomplete at the end of diastole.

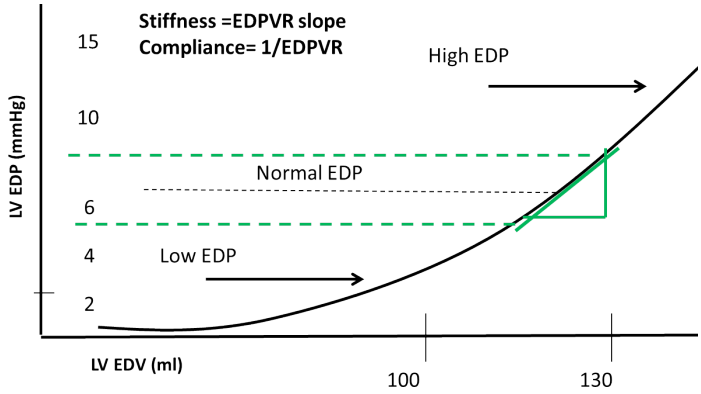

Fig. 3: Schematic drawing. EDPVR represents the relation between EDP and EDV, at the stage of the cardiac cycle that is marked by A-V (mitral) valve closure. The non-linear curve represents diastolic stiffness with the exponential fit EDP=A*exp (k*EDV), where k is diastolic stiffness constant. Since the EDPVR is nonlinear, the compliance varies with volume; compliance is greatest at low volume and smallest at high volumes.

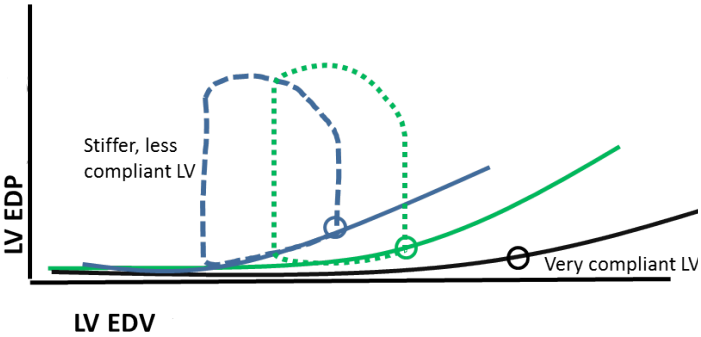

Fig. 4: Schematic drawing. EDPVR changes with lusitropic conditions. Examples of decreasing compliance detected by EDPVR leftward shift (stiffening of LV) include restrictive cardiomyopathy, infiltrative disease (amyloid), and hyperthrophic cardiomyopathies.

Understanding Lusitropy Cont.

PASSIVE DIASTOLIC PROPERTIES

- Compliance (dV/dP, inverse of stiffness): LV compliance is determined by the substantial properties of the cardiac myocytes, cardiac fibroblasts, and other cardiac cells along with their cellular-molecular preparedness to contraction and relaxation.
- Stiffness (dP/dV, inverse of compliance)
- EDPVR: LV end-diastolic pressure-volume relationship provides an indication of LV compliance during the filling phase of cardiac cycle (Fig. 4 & Fig. 6). In late diastole passive properties of LV are more prominent as compared to active relaxation.
- Capacitance: Characterizes diastolic volume at given pressure. LV chamber geometry is important determinant of capacitance and its overall compliance (Fig 5).
- As myocardium is perfused mostly in diastole, stiffness of myocardium plays role in limiting coronary perfusion (7).

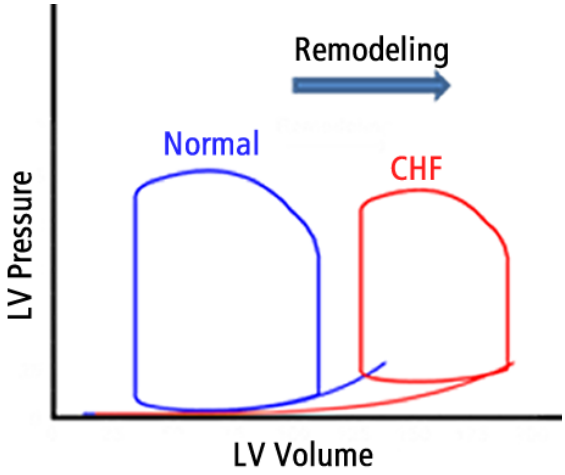

Fig. 5: Schematic drawing. Chronic heart failure (CHF) is seen in the late stages of post-myocardial infarct injury remodeling. Over time the remodeling mechanism persists beyond control and, in the non-injured region, cardiomyocytes hypertrophy and fibroblasts proliferate producing interstitial collagen. As the LV chamber volumes increase (EDV & ESV) the PV loop shifts to the right. However both SV and SW are diminished.

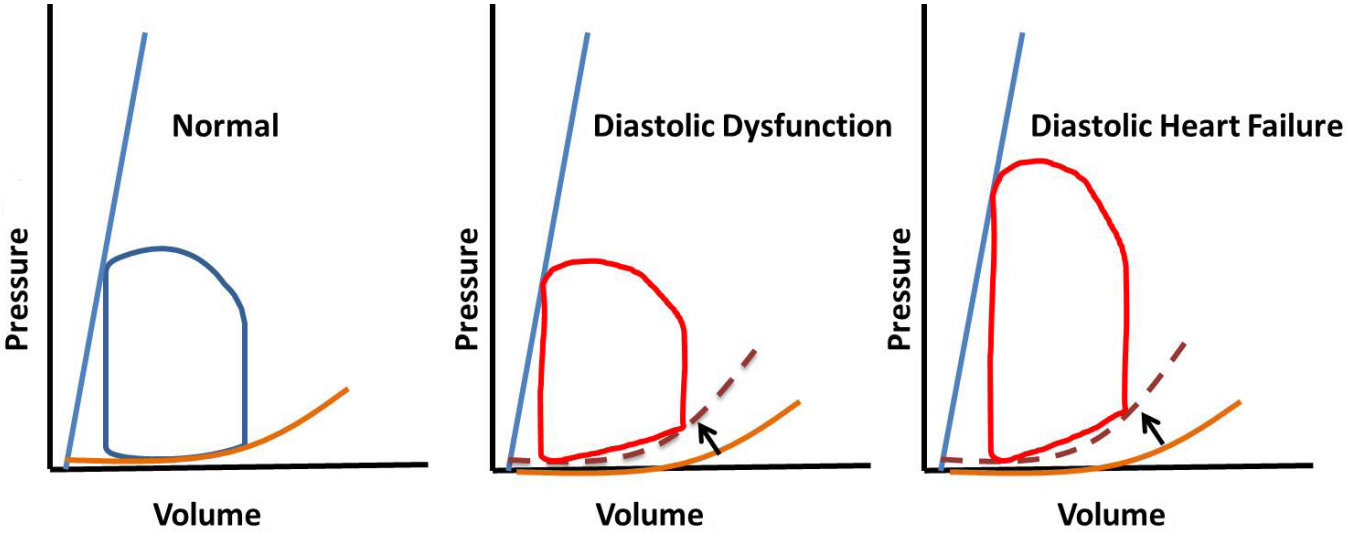

Fig. 6: Schematic drawing. Diastolic dysfunction is a syndrome characterized by impaired ventricular filling resulting from prolonged active LV myocardial relaxation and/or increased passive diastolic LV stiffness. Both indexes can help to determine diagnosis of diastolic dysfunction, and/or diastolic heart failure.

Understanding Lusitropy Cont.

Lusitropy can be further detected by non-invasive measurement of velocities of myocardial tissue using Tissue Doppler Imaging (TDI) echocardiography by Doppler E-wave deceleration time (DT) (Fig. 7). Many subjects with prolonged Tau interval (IVR) show a well delayed E-wave relaxation pattern on echocardiographic exam. However this relationship of Tau and E-wave does not always have good correlation since Tau requires a mathematical fit to the pressure contour (2).

Another method for assessing diastolic indices is speckle tracking echocardiography (STE), where myocardial speckles (small structures) are tracked to determine myocardial velocity and strain (6). Strain is the change in velocities length during a given time period, and it is possible to measure it by STE in the longitudinal, circumferential, transverse, and radial directions to assess regional diastolic function such as interstitial fibrosis in the region to identify myocardial viability.

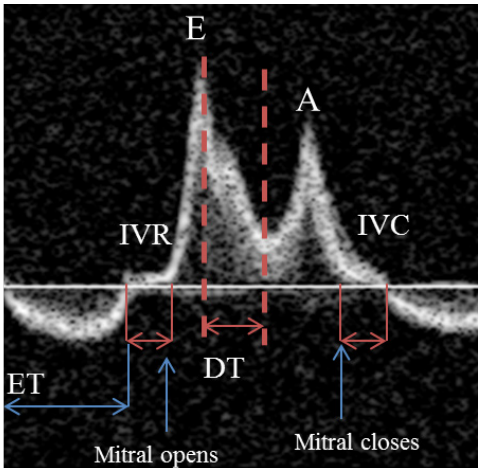

Fig. 7: E-wave deceleration time corresponds to diastolic relaxation properties. E-wave duration that is prolonged and lower in peak value than the A-wave, often represents underlying diastolic dysfunction. (IVC) Isovolumic contraction time, (DT) deceleration time, (IVR) Isovolumic relaxation time, (ET) ejection time.

REFERENCES

(1)Buckberg G, Mahajan A, Saleh S, Hoffman JIE, Coghlan C. "Structure and function relationship of the helical ventricular myocardial band." J Thorac Cardiovasc Surg 136: 578–589, 2008.

(2) Davis KL, Mehlhorn U, Schertel ER, Geissler HJ, Trevas D, Laine GA, Allen SJ. "Variation in tau, the time constant for isovolumic relaxation, along the left ventricular base-to-apex axis." Basic Res Cardiol. 1999 Feb;94(1):41-8.

(3) Weiss JL, Frederiksen JW, Weisfeldt ML. "Hemodynamic determinants of the time-course of fall in canine left ventricular pressure." J Clin Invest. 1976 Sep;58(3):751-60.

(4) Raff GL, Glantz SA. "Volume loading slows left ventricular isovolumic relaxation rate. Evidence of load-dependent relaxation in the intact dog heart." Circ Res. 1981 Jun;48(6 Pt 1):813-24.

(5) Matsubara H, Takaki M, Yasuhara S, Araki J, Suga H. "Logistic time constant of isovolumic relaxation pressure-time curve in the canine left ventricle. Better alternative to exponential time constant." Circulation. 1995 Oct 15;92(8):2318-26.

(6) Hoit BD. "Strain and strain rate echocardiography and coronary artery disease." Circ Cardiovasc Imaging. 2011;4:179 –190.

(7) Watanabe J, Levine MJ, Bellotto F, Johnson RG, Grossman W. "Left ventricular diastolic chamber stiffness and intramyocardial coronary capacitance in isolated dog hearts." Circulation. 1993 Dec;88(6):2929-40.

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Equipment & Best Practices

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Transonic Scisense PV Systems & Catheters

ADV500 PRESSURE-VOLUME MEASUREMENT SYSTEM

- Measure volume using either Conductance or Admittance modes
- True volume in real-time using Admittance mode
- Generate full hemodynamic reports and calculate measurements of contractility and stiffness
- Two pressure inputs for pressure gradient or pulse-wave velocity
- Compatible with all data acquisition systems (± 5V range required)

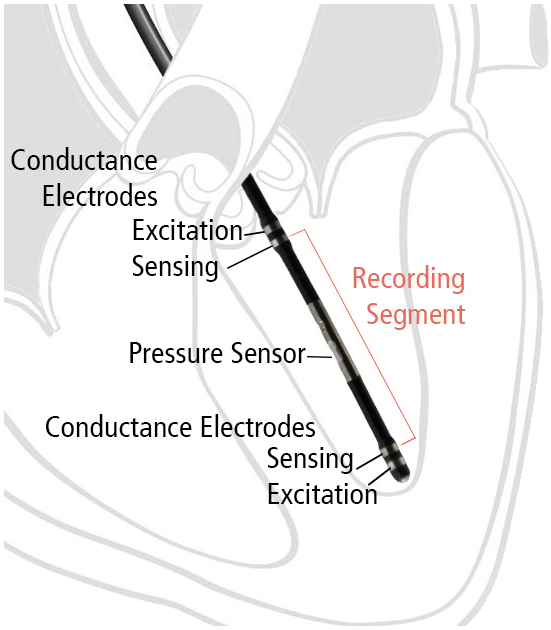
PRESSURE-VOLUME CATHETERS

Pressure-Volume Catheters are available for all animal models. 1.2F and 1.9F Catheters are ideal for mouse and rat studies, 3.5F for rabbits, and 5.0F or 7.0F for all larger animals (canine, swine, bovine, etc.)

- Excellent response at high frequencies such as rodent heart rates.
- Smooth, flexible tubing allows easy insertion and navigation.
- Catheter tips can be customized to enable easy insertion.
- Second pressure sensor available for 5.0F & 7.0F Catheters.

VARIABLE SEGMENT LENGTH (VSL) CATHETERS

VSL Catheters have 4 volume electrode options designed to offer flexibility and ensure proper fit. VSL Catheters are standard for all larger animal Catheters ranging from 3.5F - 7.0F, and optional for 1.2F and 1.9F sizes. Standard segment spacings are available for all Catheter sizes while custom designs can be made to order. Active segment can be changed at anytime during data collection.



Outer excitation electrodes create the electrical field which is detected by the inner sensing electrodes.

1.9F Rat Pressure Volume Catheter &
1.2F Mouse Pressure Volume Catheter

5F VSL Large Animal Pressure Catheter

7F VSL Large Animal Pressure Catheter
with pigtail tip

*Note: Catheters not to scale

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Research Equipment Sources

Additional equipment for surgical procedures may be acquired from the vendors listed below or from your vendor of choice.

VENTILATORS

CWE, Inc.
www.cwe-inc.com

Harvard Apparatus, Inc.
Holliston, MA
ww.harvardapparatus.com

Kent Scientific Corp. (Rattus)
Torrington, CT
www.kentscientific.com or
www.rattus.com

MICROMANIPULATORS / STANDS

Fine Science Tools, Inc.
Foster City, CA
www.finescience.com

Techni-Tool Inc.
Worcester, PA
www.techni-tool.com

Stoelting Co.
Wood Dale, IL
www.stoeltingco.com

LANGENDORFF APPARATUS

Hugo Sachs Electronik: Harvard Apparatus
Holliston, MA
ww.harvardapparatus.com

Rattus (Kent Scientific)
Torrington, CT
www.kentscientific.com or
www.rattus.com

Radnoti Glass Technology, Inc.
Monrovia, CA
www.radnoti.com

INFUSION PUMPS

Razel Scientific Instruments, Inc.
Stamford, CT
www.razelscientific.com/

Rattus (Kent Scientific)
Torrington, CT
www.kentscientific.com or
www.rattus.com

SONOMICROMETERS

Sonometrics Corporation
London, Ontario, Canada
www.sonometrics.com

FLUID INFUSION

Instech Laboratories, Inc.
Plymouth Meeting, PA
www.instechlabs.com

SURGICAL MONITORING AND HEATING

Indus Instruments
Webster, TX
www.indusinstruments.com

SURGICAL SUPPLIES

Covidien (Kendall)
Mansfield, MA
www.covidien.com
www.kendallhq.com

BD
Franklin Lakes, NJ
www.bd.com

Harvard Apparatus
Holliston, MA
ww.harvardapparatus.com

CLEANING / DISINFECTING AGENTS

Alconox Inc.
White Plains, NY
www.alconox.com

Ruhof
Mineola, NY
www.ruhof.com

Advanced Sterilization Products (J & J)
Irvine, CA
www.aspjj.com

How to Collect Quality PV Data

There are three main steps which need to be followed in order to maximize your chances of collecting quality PV data. They are:

1. Proper system set-up and calibration
2. Careful catheter positioning using pressure, magnitude and phase signals
3. Thoughtful data collection and analysis

1. System Set-up

Turn on system and start catheter hydration → Enter values for Admittance calibration factors:
- Stroke volume
- Blood resistivity
- Heart type

→ Balance pressure sensor

2. Catheter Positioning

Place catheter into ventricle → Position catheter in center of ventricle by viewing Pressure vs. Magnitude (Conductance) loops and phase signal → Once optimal signals are achieved, press "Enter" during acquisition to perform a baseline scan

Accept the reported scan numbers, or rescan if needed

3. Data Collection and Analysis

Switch to Pressure vs. Volume loops view → Continue with protocol steps (injections, occlusions, etc.) → Use software analysis features to study hemodynamic results

ADV500 Quick Start Guide - Initial Setup

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

1. Basic Hardware Connection

Find a suitable location near work station or on roll cart (not provided). ADV500 Pressure-Volume 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 Sub-D cable ② to Data Output ②
- Connect HDMI cables ③ to Catheter Inputs ③

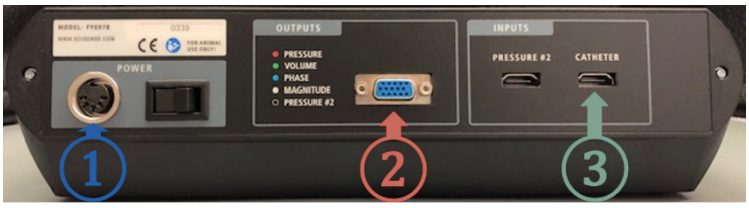

Fig. 1: ADV500 rear. Use in conjunction with Fig. 2

Fig. 2: ADV500 cables. Use in conjunction with Fig. 1

2. System Power Up

Using power switch on rear of ADV500, turn on the system and verify that the splash screen is displayed (Fig. 3).

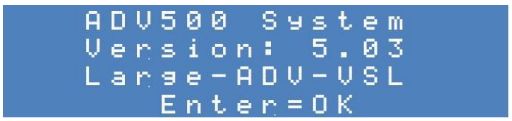

Fig. 3: Welcome splash screen. Shown here is a large animal firmware license – your license may be different depending on what version was purchased.

Press "Enter" to move to main menu.

Note: When there is no catheter connected to the ADV500, the 'No Catheter Menu' will appear (Fig. 4).
When a catheter is connected to the ADV500, the 'Catheter Menu' will appear (Fig. 5).

Fig. 4: No Catheter Menu

Fig. 5: Catheter Menu

ADV500 Quick Start Guide - Initial Setup Cont.

Fig. 6: ADV500 integration with DAQ

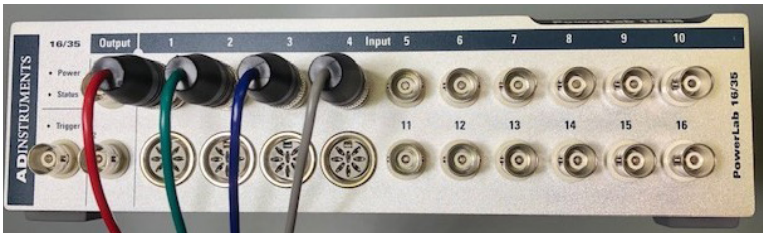

Fig. 7: ADI PowerLab A-D Converter DAQ

Note: "Pressure #2" input is only used if a secondary pressure catheter is being utilized. It is not needed for PV loop data collection.

3. Data Acquisition Integration

Connection to a Data Acquisition System (DAQ) can be made using the 5 BNC connectors located on the Sub-D output cable (See ② in Fig. 2). Using the guide provided on the back of the ADV500 (see Table 1) attach the BNC connections to input channels of the DAQ in the corresponding order.

Table 1:

DAQ CHANNEL	COLOR	FUNCTION
1	Red	Pressure
2	Green	Volume
3	Blue	Phase
4	Gray	Magnitude
5	Black	Pressure #2

4. Template File Setup

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 all output channels provided by the ADV500.

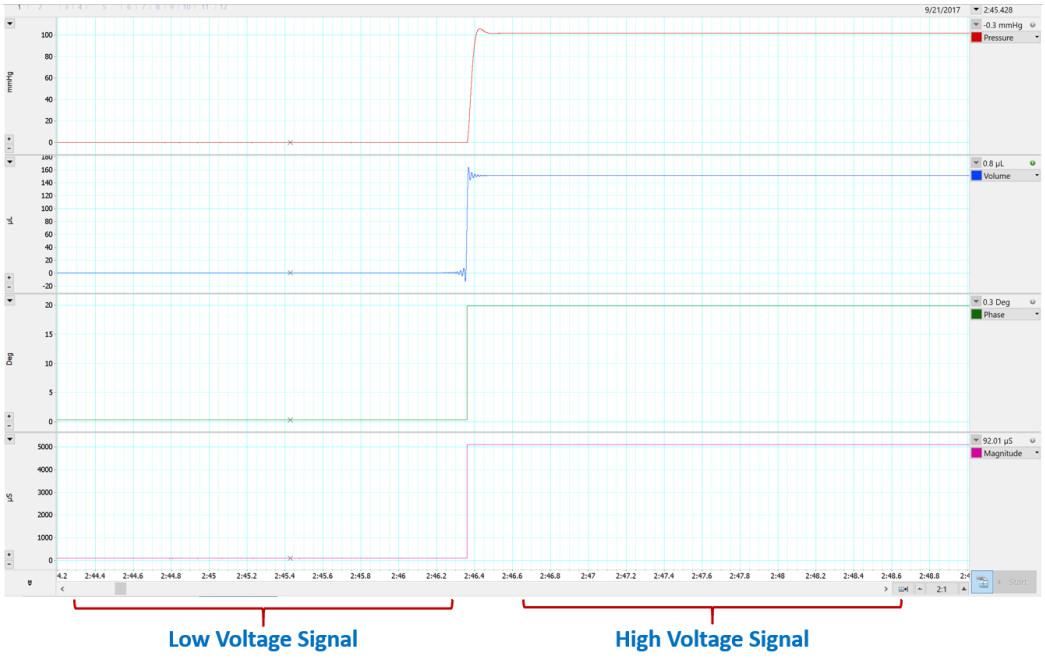

Fig. 8: Left flat line represents the low voltage signal coming from the ADV500 while the right flat line represents the high voltage signal. These will be used for unit conversion in the software.

ADV500 Quick Start Guide - Initial Setup Cont.

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. From the "Catheter Menu" of the ADV500, select "System Settings" and then "Send Cal. Signals." This will send out low voltage reference signals for display on your data acquisition system (Fig. 8).

c. Press "ENTER" on the ADV500 to switch to the high calibration output. You should see a jump in your data at this moment on your DAQ (Fig. 8).

d. At this stage you can stop data recording on your software and open the calibration window for each data channel. You will be asked to input conversion data for both the low and high signals sent by the ADV500 – you can also use this as an opportunity to name your software channels and provide them with the correct units. The calibrated output values and corresponding units for the low & high signals are shown on the LCD screen of the ADV500 during low & high output and in Table 2. Consult your DAQ's operating manual for more information related to voltage conversion.

Note: Data output depends on catheter French size, if using different catheter sizes in your lab, a template file will need to be established for each size.

Table 2:

ADV500 OUTPUT	DAQ INPUT	VOLTAGE OUTPUT FROM ADV500		CATHETER SIZE: 1.2F		CATHETER SIZE: 1.9F		CATHETER SIZE: 3.5F		CATHETER SIZE: 5F & 7F	
		LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
Pressure (mmHg)	1	~ -2.87 Volts	~ -0.58 Volts	0 mmHg	100 mmHg	0 mmHg	100 mmHg	0 mmHg	100 mmHg	0 mmHg	100 mmHg
Volume (µL or mL)	2	~ 0 Volts	~ 4.9 Volts	0 µL	150 µL	0 µL	1000 µL	0 mL	4 mL	0 mL	300 mL
Phase (degrees)	3	~ 0 Volts	~ 4.9 Volts	0°	20°	0°	20°	0°	20°	0°	20°
Magnitude (µS or mS)	4	~ 0 Volts	~ 4.9 Volts	0 µS	5000 µS	0 µS	5000 µS	0 mS	15 mS	0 mS	50 mS
Pressure 2 (mmHg)	5	~ -2.87 Volts	~ -0.58 Volts	0 mmHg	100 mmHg	0 mmHg	100 mmHg	0 mmHg	100 mmHg	0 mmHg	100 mmHg

Note: If you are using a Variable Segment Length (VSL) catheter, the high calibration for volume may deviate from the chart above. Consult the ADV500 splash screen when sending high outputs to confirm or consult your local representative. VSL catheters are available for catheters 1.9F and larger.

e. Confirm the sampling rate of your DAQ and adjust as needed. We suggest 1KHz for rodent models and 200Hz for larger animals.

f. Once complete, it is essential that you save the settings of this file within your software for daily data recording. Most software providers distinguish between "save as type," or the type of file you want to save, be sure to save the settings of this file and not the data. You will open this settings file daily and therefore avoid the necessity to repeat these software setup steps in the future.

ADV500 Quick Start Guide - Daily Use

THIS GUIDE WILL COVER THE SUGGESTED DAILY USE STEPS FOR YOUR ADV500 PV EQUIPMENT

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). 3.5-7F catheters can be placed in 50 mL Falcon/BD tubes. The catheter does not need to be plugged into the ADV500 for this process.

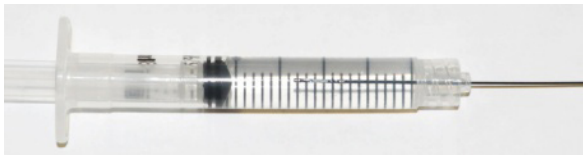

Fig. 1: Catheter hydrating in a syringe

2. Turn ADV500 and PV Catheter ON

Using the provided HDMI cables, plug one end of the HDMI cable into the catheter and the other end into the "Catheter" input on the back of the ADV500. If using a second pressure catheter, repeat this process using the "Pressure 2" input. Power on ADV500 using the power switch on the back.

Press "Enter" on keypad to move from the welcome screen to the "Catheter Menu." Menu options can be navigated using the up and down arrows located above the "Enter" button on the keypad. The catheter can be turned on by pressing "Enter" on "Acquire Data" (Fig. 2). All options in the menu can be selected by navigating to them and pressing "Enter" or alternatively, you can press the corresponding number on the keypad.

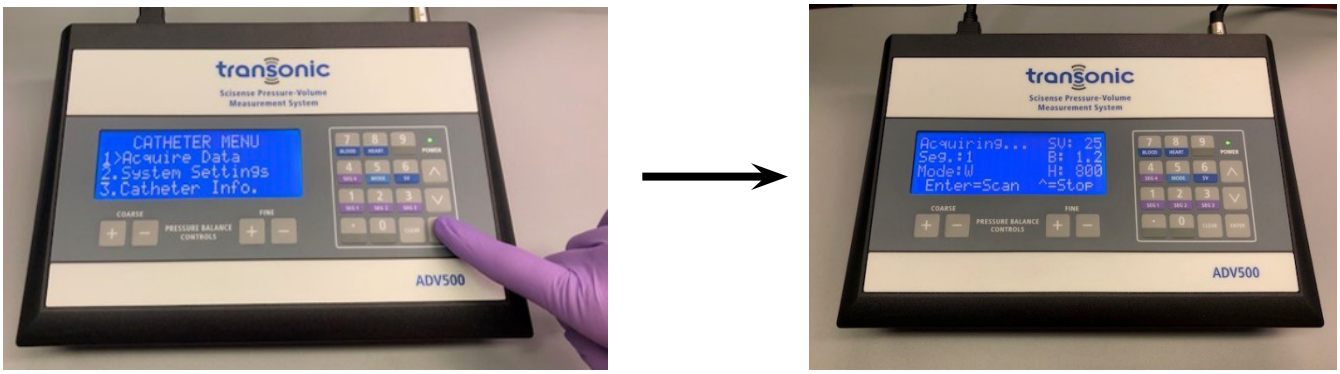

Fig. 2: Selecting "Acquire Data" from the catheter menu turns the catheter on.

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

Template settings files are matched to corresponding catheter size – be sure you select the settings file that matches the French size of your hydrating catheter (1.2F, 1.9F, 3.5F, 5F or 7F).

Load your software program and select your pre-saved template file. Refer to "Step 4" of our "ADV500 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 – data should start to move across your screen.

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ADV500 Quick Start Guide - Daily Use Cont.

4. Balance Pressure Sensor

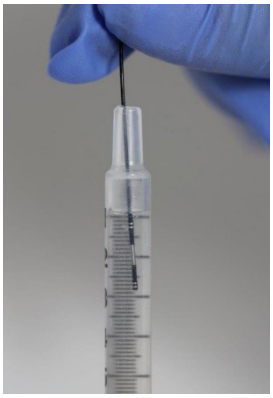

Fig. 3: Catheter balance below water line

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. 3).

Using the "Pressure balance controls" on the ADV500 hardware and the "Pressure" channel on your DAQ as reference, bring the recorded pressure value to 0mmHg. This can be accomplished using the "COARSE" and "FINE" adjustment buttons below the LED screen of the ADV500 (Fig. 4).

> 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.

5. Confirm Study Parameters Are Input Correctly

Stroke volume, Heart Type and Blood Resistivity are all important parameters to consider for accurate data collection. Each of these parameters can be adjusted from either the "Catheter Menu" or most conveniently through the main acquire data collection screen (Fig. 5, red) using quick access buttons on the Keypad (Fig. 5, green). Below is a review of how to adjust each parameter:

Fig 4: Pressure balance controls

Stroke Volume: This can be taken from prior echo measurement, prior experimental data or approximation. To change this variable from the acquire screen, press #6 on the keypad, type in your new value and press "Enter" to return to acquiring data.

Heart Type: For this variable we have pre-selected values already stored in the ADV500 – "Normal" and "Infarcted." If you are working with Myocardial Infarction models, press #8 on the keypad, select "Infarcted" and press "Enter." All other animal models can utilize the "Normal" mode.

Blood Resistivity: This variable is largely unchanged for a very wide array of applications and can generally be left in the 1.2-1.6 range that automatically populates. If a change is required, press #7 on the keypad, enter your new value and press "Enter."

> Note: "Mode" should always be "W." "D" mode is not validated for all protocols and therefore not suggested at this time.

Fig 5: Highlighted in red is the main data collection screen while green highlights the quick access buttons on the keypad

6. Insert Catheter Through Right Carotid, Femoral Artery or Apex

Please refer to our technical note, "Optimizing Catheter Life Span," for tips and tricks to extend the life of your catheter. A selection of surgical applications - including pictures and detailed instructions - are available in your original shipment, through our website or your local representative.

> Note: We suggest apical insertion for all rodent right ventricular applications.

7. Collect Data

See our "ADV500 Quick Start Guide – Data Collection" available in your original shipment, through your local representative or through our website.

8. Remove Catheter and Clean

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

ADV500 Quick Start Guide - Data Collection

THIS GUIDE WILL COVER TIPS AND TRICKS FOR PROPER DATA COLLECTION USING YOUR ADV500 PV EQUIPMENT

1. Monitor Pressure Signal Only

For retrograde LV catheter insertions (Carotid, Femoral) you will see a systemic pressure signal initially (Fig. 1A). Advance the catheter forward, as you get close to the aortic valve you will start to see a dicrotic notch in the pressure signal (Fig. 1B). Using very small movement, move forward and backwards until the catheter enters the ventricle where the pressure change will be dramatic (Fig. 1C) – do not force this process, patience here is essential. For apical insertions – optional for LV applications but required for all rodent RV applications – you will immediately see ventricular pressures. (Fig. 1D). General LV pressure ranges for various models can be found in Table 1.

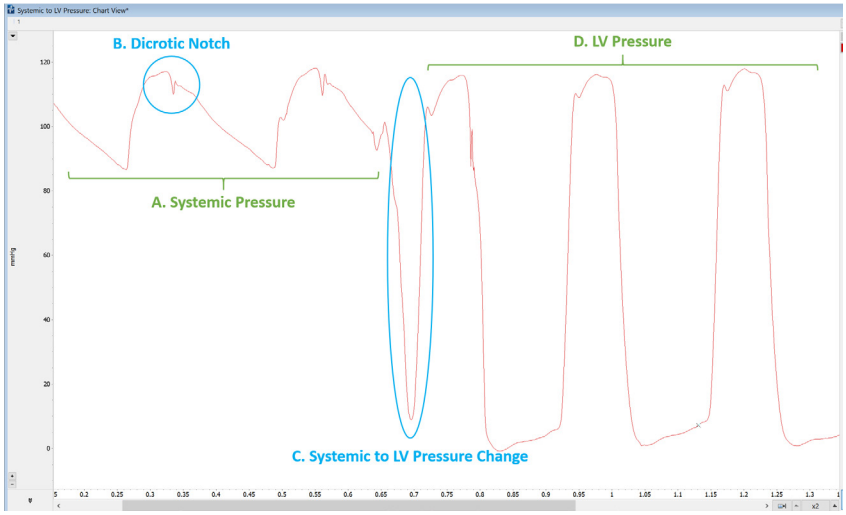

Fig. 1: Example of systemic pressure, LV pressure and the transition between the two as the catheter moves through the aortic valve. Also pictured is the dicrotic notch which helps to identify proximity to the heart.

2. Mapping Pressure versus Magnitude ("P v M") on the XY Plot

Though volume is the goal, we first want to confirm proper catheter placement - monitoring the overall shape and width of the P v M loop will help to achieve this. For LV work, the shape of the loop will be roughly rectangular while RV work can yield a few different general shapes (Fig. 2). The key variable at this step will be the width of the loop with the goal being to get the loop as wide as possible. Suggested minimum range for the magnitude for common animal models can be found in Table 1.

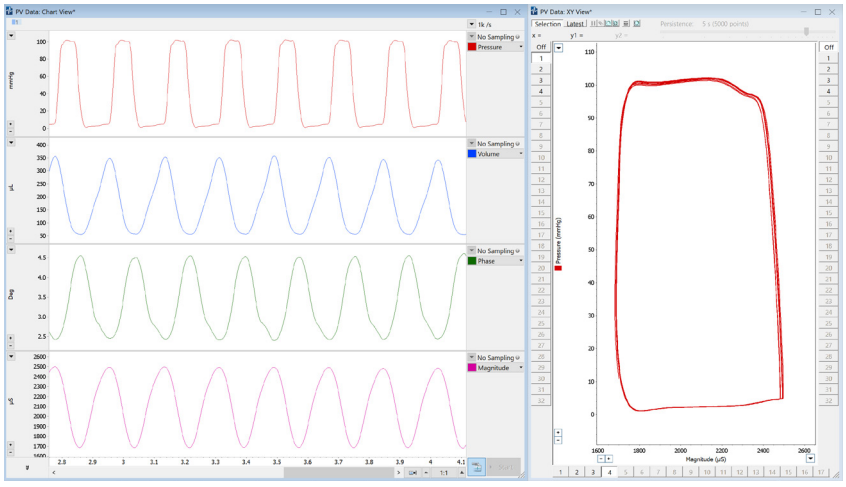

Fig. 2: Pressure versus Magnitude raw data collection and expected general LV shape

Table 1	MOUSE	RAT	DOG/PIG/SHEEP
Systolic Pressure (mmHg)	90-120	100-130	70-100
Diastolic Pressure (mmHg)	1-6	1-6	1-6
Heart Rate (BPM)	> 450	> 350	> 50
Magnitude Variation	≥ 300uS	≥ 500uS	≥ 2.5mS
Phase Variation (Degrees)	2-8	2-7	1-5

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ADV500 Quick Start Guide - Data Collection Cont.

3. Phase Can Begin to be Monitored

Once P v M loops fall into a correct shape and range, you will want to look at phase. Phase represents the muscle's contribution to the total signal and the higher the number, the more muscle present. We are looking for the minimum muscle contribution achievable because this indicates the catheter is localized in the middle of the heart, which is essential. A typical phase signal can be found in Figure 3. See Table 1 for expected ranges in various animal models.

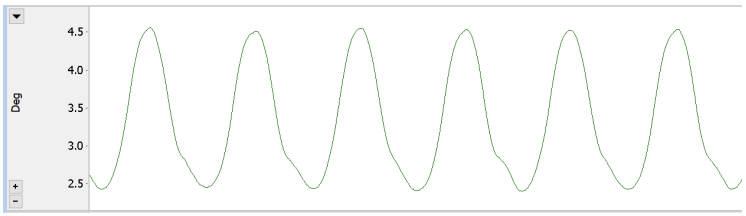


Fig. 3: A typical phase signal is sinusoidal in shape.

4. A "Baseline Scan" Can Now be Completed to View a Corrected Volume

In general terms, the ADV500 calculates volume by taking magnitude (total blood and muscle signal) and deletes Phase (muscle only signal) to give us blood conductance only. This then gets processed inside of the hardware to yield an absolute volume based on current catheter position. To accomplish this, you will press the "Enter" button on the ADV500 and allow it to complete its scan (Fig. 4). Accept the values to see an updated volume based on your catheter position.

Note: If catheter position changes during your protocol as a result of manual manipulation or other factors, a baseline scan should be repeated to ensure signal fidelity.

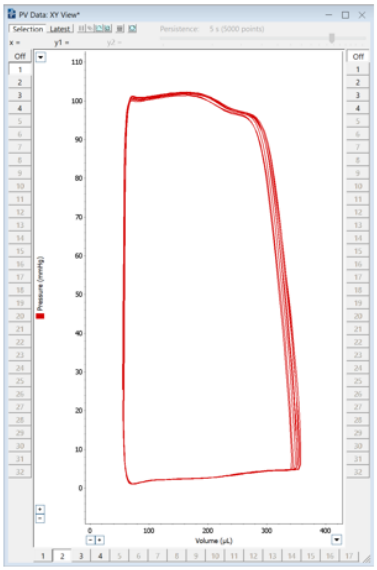
Fig. 4: A baseline scan is performed by pressing "Enter" during data collection. This process allows for volume to be calculated accurately.

ADV500 Quick Start Guide - Data Collection Cont.

5. Confirm the volume signal fidelity – start to map Pressure versus Volume (P v V) on the XY Plot

The volume measured from the heart directly correlates to catheter position, therefore confirmation of signal fidelity is an important final step in this process. Use your recording software to calculate common variables like cardiac output, stroke volume and ejection fraction (Fig. 5). These values should look physiological and correlate with other modalities, if used. If volume does not look appropriate in either shape or absolute values, reposition the catheter as described starting again at step 2, proceeding back through to step 5.

6. Complete protocol design



	CO (V/min)	SV (V)	Vmax (V)	Vmin (V)	Ves (V)	Ved (V)	Pmax (V)	Pmin (V)	Pmean (V)	Pdev (V)	Pes (V)	Ped (V)	HR (bpm)	EF (%)
1	9.05E+04	270	370.3	60.1	64.02	365.4	103.8	1.11	39.84	102.7	99.11	5.17	335.2	82.91
2	9.56E+04	283.7	376.5	58.57	65.49	372.2	103.8	1.18	40.33	102.6	100.1	5.24	337.1	83.63
3	9.20E+04	276.1	386.3	59.82	64.84	373.3	103.9	1.19	40.05	102.7	100	5.05	333.3	83.26
4	9.79E+04	292.2	394.5	60.33	66.65	388.3	104.3	1.34	40.64	103	101	5.51	335.2	83.7
5	9.00E+04	265.5	363.4	59.68	64.9	361.7	103.8	1.01	40.42	102.7	100.9	5.17	339	82.66
6	8.40E+04	252.1	351.2	56.41	62.46	336	103	1.08	39.22	101.9	97.25	4.96	333.3	82.48
7	8.81E+04	261.4	354.1	56.94	62.35	352.2	102.9	1.08	39.77	101.8	99.57	5.29	337.1	83.19
8	8.72E+04	258.7	370.5	57.11	63.53	352.3	102.6	1.18	39.53	101.5	98.19	5.02	337.1	81.96
9	9.09E+04	269.5	373.3	60.18	65.62	371.6	102.9	1.11	39.84	101.8	98.76	5.21	337.1	81.9
10	9.20E+04	273.1	371.3	58.9	64.95	365.8	102.6	1.01	39.53	101.6	98.61	4.98	337.1	83.28
11	8.77E+04	261.5	355.5	58.22	63.27	352.9	102	1.03	38.87	101	97.7	4.81	335.2	82.72
12	8.89E+04	265.2	356.1	56.52	62.9	351.2	101.7	1.06	38.74	100.6	96.74	4.89	335.2	82.58
13	8.75E+04	259.5	350.4	57.07	62.75	348.3	101.6	1.04	38.99	100.6	97.52	5	337.1	82.71
14	8.80E+04	262.4	353.3	56.35	63.27	349	101.7	1.1	38.91	100.6	97.11	5	335.2	82.34
15	8.73E+04	259	352	57.28	62.74	348	101.8	1.08	39.22	100.7	96.69	5.11	337.1	82.76
16	8.86E+04	262.9	355.9	56.08	63.01	353.6	101.9	1.08	39.29	100.8	97.11	5.1	337.1	83.08
17	8.55E+04	255	348.3	57.36	64.3	346.4	101.9	1.11	39.14	100.8	98.46	5.1	335.2	82.34
18	8.99E+04	265.1	353.7	56.36	62.87	349	102.1	1.08	39.7	101	96.25	5.15	339	83.15
19	8.65E+04	258	353.9	57.09	63.76	349.2	102.2	1.14	39.3	101.1	97.55	4.87	335.2	82.38
20	8.85E+04	264	357.7	56.84	64.1	355.2	102.3	1.07	39.33	101.2	97.86	5.11	335.2	83.04
21	8.80E+04	258.1	351.7	56.68	63.84	350.3	102.1	1.04	39.8	101	97.22	5.17	340.9	82.73
22	8.43E+04	251.5	344.5	56.24	63.41	341.6	101.7	1	38.8	100.7	96.1	4.73	335.2	82.31
	CO (V/min)	SV (V)	Vmax (V)	Vmin (V)	Ves (V)	Ved (V)	Pmax (V)	Pmin (V)	Pmean (V)	Pdev (V)	Pes (V)	Ped (V)	HR (bpm)	EF (%)
Avg	8.90E+04	264.7	361.1	57.73	63.86	356.1	102.6	1.096	39.51	101.5	98.17	5.075	336.3	82.78
Min	8.40E+04	251.5	344.5	56.08	62.35	336	101.6	1	38.74	100.6	96.1	4.73	333.3	81.9
Max	9.79E+04	292.2	394.5	60.33	66.65	388.3	104.3	1.34	40.64	103	101	5.51	340.9	83.7
Count	22	22	22	22	22	22	22	22	22	22	22	22	22	22

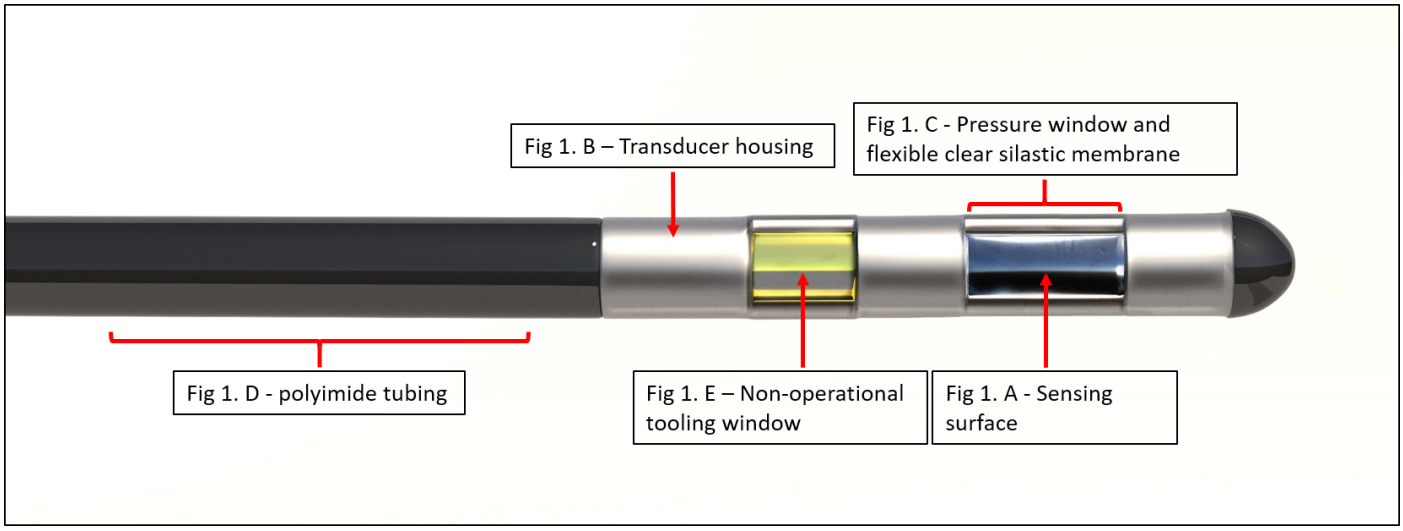
Fig. 5: Confirmation of signal volume fidelity is an important final step in catheter positioning. Cardiac Output, Stroke Volume and Ejection Fraction are commonly used for verification.

If the volume signal achieved in step 5 is acceptable, run your protocol as designed through to completion. Return to step 8 of our "ADV500 Quick Start Guide – Daily Use" once protocol is completed.

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:

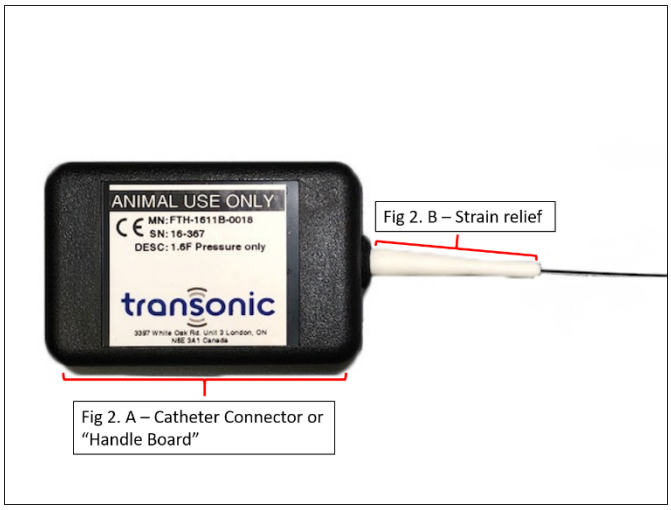
In order to prolong the life of a micro catheter, it is important that the user have some appreciation of the mechanism. The sensing surfaces, tube wall and conducting wires are measured on the order of microns. It is not unrealistic for an untrained user to unknowingly apply damaging forces to the catheter.

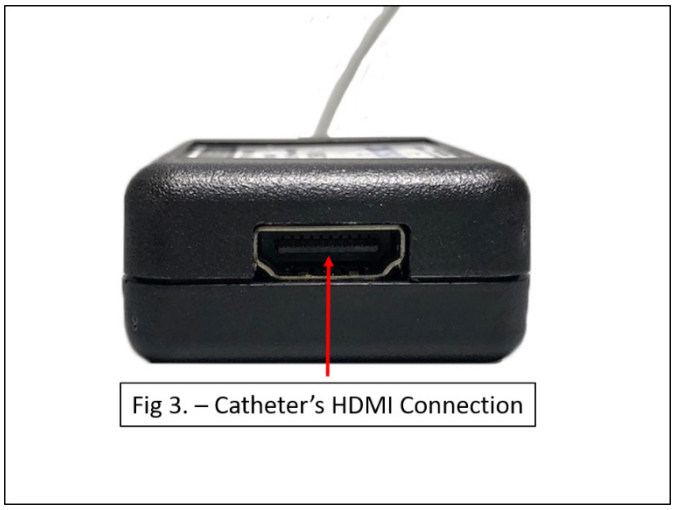
1. A common form of damage to micro tip catheters is catheter shaft damage from excess force applied to the shaft. Similar to an eggshell, the polyimide shaft material will collapse if it is deformed past a certain point either by bending or crushing. Shaft strength will not return once it has gone past 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.



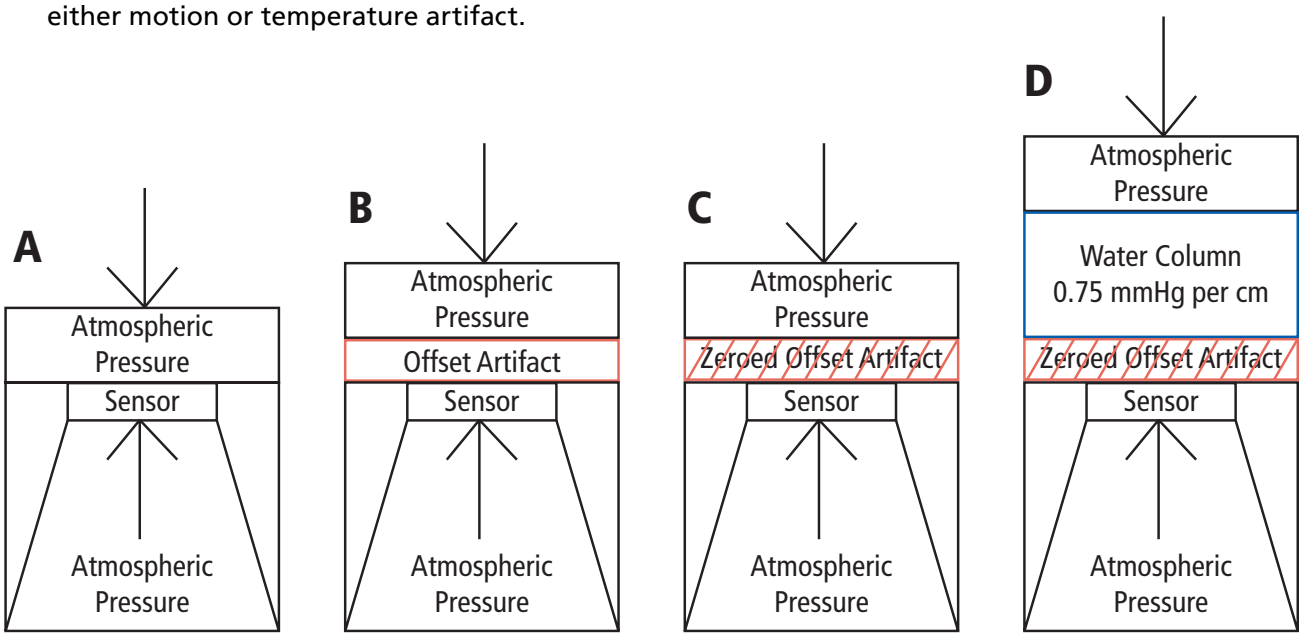


Balancing Pressure Sensors Before Use

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.

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.

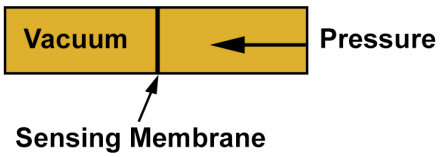
Pressure Sensing Technology Basics

- 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 129 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

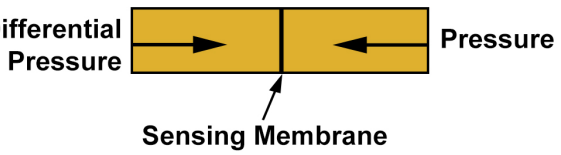
ABSOLUTE PRESSURE SENSOR:

The device measures pressure relative to internal reference vacuum.



DIFFERENTIAL PRESSURE SENSORS:

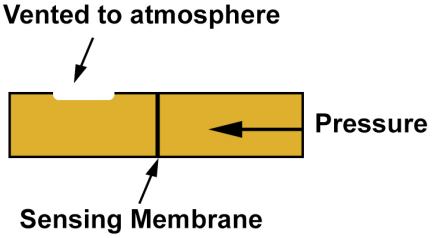
The device measures difference between two pressure measurements placed on the same sensing structure. Instead of measuring relative to atmospheric pressure, differential measurements are taken with respect to a specific reference pressure.



GAUGE PRESSURE SENSOR:

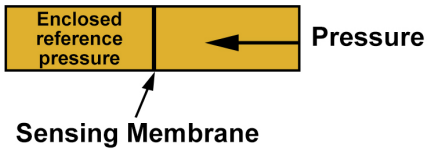
The device measures relative to atmospheric pressure, part of the sensor must be “vented” to atmosphere.

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:

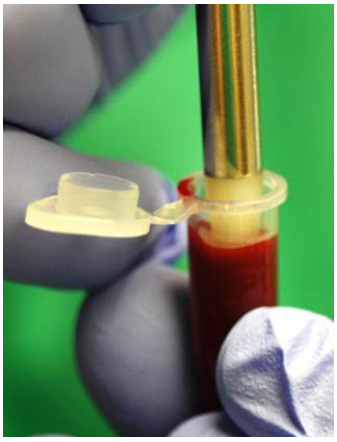
The device senses pressure relative to some permanent reference pressure inserted before sealing (It functions as a differential pressure sensor).



Measuring Blood Resistivity with a Calibration Probe

The Calibration Probe measures electrical properties of blood. The resistivity (ρ) measurement in ohm meter (Ωm) is later inputted into ADV500 and is used when pressure-volume parameters are calculated.

BLOOD RESISTIVITY (ρ) MEASUREMENT



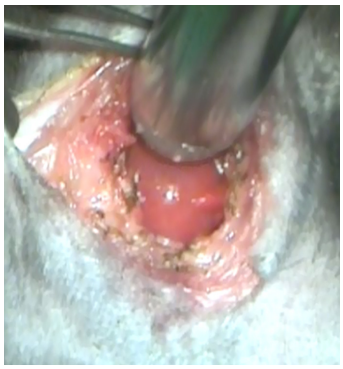
Calibration Probe is used to measure blood resistivity.

1. To measure blood resistivity, connect the Calibration Probe to the HDMI cable connected to ADV500 CATHETER input slot on the back of the unit.
2. The CAL. PROBE MENU will emerge on the front screen. Two selections will appear:
   a. Acquire Data
   b. Probe Information
3. Select 1 (Acquire Data)
4. The CALIBRATION MENU with 3 choices will appear:
   a. Blood Properties
   b. Muscle Properties
   c. Previous Menu
5. Select 1 (Blood Properties)
6. Draw blood from one animal into a 0.5 ml cryogenic vial, obtaining as much as possible in order to ensure a good measurement. Do not heparinize the blood for this measurement.
7. Do the measurement immediately after blood is drawn from the animal. Ensure that the blood is not clotting when the measurement is taken.
   - When measuring, please maintain blood at approximately 37°C. Reduce heat loss as much as possible. Note that the resistivity measured by the Calibration Probe will be 2.1% higher for each additional degree of temperature, or 2.1% lower for each 1 degree decrease in temperature.
8. Place the Calibration Probe tip just below the meniscus on the surface of blood collected in the vial and press "ENTER" to calculate the blood resistivity in units of Ωm.
9. If possible, repeat this procedure on 3-4 animals with similar genetic background that will be used for the study to obtain a mean (ρ) value.
10. The value for blood resistivity will be displayed on the screen and should lie between 1.0 and 2.0 Ωm for most species. Press "ENTER" to accept the value or press ↑ to recalculate. Record the mean (ρ) value.
11. Later, the Pressure-Volume Catheter is connected to ADV500 CATHETER input slot on the back of the unit, replacing the Probe. In the CATHETER MENU, the default value for blood resistivity, can be substituted by the mean resistivity value from the Calibration Probe measurements. This BLOOD (ρ) value can be found in the CATHETER MENU > System Setting > Study Parameters > Blood (ρ).

Measuring Muscle Properties with a Calibration Probe

The Calibration Probe or Epicardial Surface Probe measures electrical properties of myocardium. The ratio of Sigma/Epsilon (σ/ε) is later inputted into ADV500 and is used when pressure-volume parameters are calculated.

SIGMA/EPSILON (σ/ε) MEASUREMENT



Calibration Probe measuring ratio of heart muscle conductance to permittivity (σ/ε) in a mouse.

1. To measure the muscle properties of the myocardial wall, connect the Calibration Probe to the HDMI cable connected to the ADV500 CATHETER input slot on the back of the unit.
2. The CAL. PROBE MENU will emerge on the front screen. Two selections will appear.
   a. Acquire Data
   b. Probe Information
3. Select 1 (Acquire Data)
4. CALIBRATION MENU with 3 choices will emerge.
   a. Blood Properties
   b. Muscle Properties
   c. Previous Menu
5. Select 2 (Muscle Properties)
6. Lower the Probe and position it on the moist epicardial heart surface away from any major vessels. Verify contact of Calibration Probe with surface of the myocardium by read-through numbers for Resistivity and Conductivity/Permittivity (σ/ε) ratio on the screen.
   - It is advisable to use a micromanipulator to obtain more controlled and consistent results from the Calibration Probe.
7. The calculated muscle properties: Resistivity (in ohm meter) and the ratio of Conductivity/Permitivity (σ/ε) in (K/s) will be displayed on the screen. The value of σ/ε should be of the order of 700,000 to 1,200,000 for normal hearts. Write down the σ/ε ratio, the second number, and repeat the measurement at least 3 times and take mean value of the measurements. Press "ENTER" to accept the value or press ↑ to recalculate.
8. Later, the Pressure-Volume Catheter is connected to the ADV500 CATHETER input slot on the back of the unit, replacing the Calibration Probe. In the CATHETER MENU, measured value of Sigma/Epsilon (σ/ε) is known as HEART TYPE and can be substituted by value obtained by measurement under "Custom". This "Custom" HEART TYPE value that is default 800K for normal hearts can be found in the CATHETER MENU > System Setting > Study Parameters > Heart Type.

DEFAULT VALUES FOR THE ADV500		
SPECIES	**BLOOD RESISTIVITY**	**HEART TYPE σ/ε**
Mouse	1.2 Ωm	800,000 or 900,000 /s
Rat	1.4 Ωm	800,000 or 900,000 /s
Rabbit	1.5 Ωm	800,000 or 900,000 /s
Dog	1.5 Ωm	800,000 or 900,000 /s
Swine	1.5 Ωm	800,000 or 900,000 /s
Sheep	1.5 Ωm	800,000 or 900,000 /s
Cow	1.5 Ωm	800,000 or 900,000 /s

Note: For the Heart Type values. 800,000 is for normal hearts and 900,000 is for infarcted hearts. Custom values may also be used.

Measuring Muscle Properties without a Calibration Probe

The Admittance derived volume technique requires the knowledge of the ratio of muscle conductance to permittivity (σ/ε). Currently this measurement is obtained by placing a surface calibration probe in direct contact with the myocardium.

This process is both error prone (poor reproducibility) and invasive. This step in the system calibration makes the current ADVantage system less attractive to researchers and may prevent its adoption in clinical settings.

The following note explores the possibility of measuring the muscle properties directly from the Admittance Catheter itself. Intuitively one can expect that if the Admittance Catheter can observe the effects of the myocardium, then it can also measure it. The theory of how this might be achieved is explained below.

Consider the complex admittance as given by:

$$Y(G_b, C_m, \omega) := \left[G_b \quad \overline{\frac{\sigma}{\varepsilon}} \cdot i\omega \quad C_m\right]$$

This value can be measured at different frequencies simultaneously to isolate C_m:

$$C_m\cdot\left(\frac{\sigma}{\varepsilon}+i\cdot\omega\right)_1 - C_m\cdot\left(\frac{\sigma}{\varepsilon}+i\cdot\omega\right)_2 = Y(G_b, C_m, \omega_1) - Y(G_b, C_m, \omega_2)$$

$$C_m\cdot i\cdot(\omega_1-\omega_2) = Y(G_b, C_m, \omega_1) - Y(G_b, C_m, \omega_2)$$

$$C_m = \frac{Y(G_b, C_m, \omega_1) - Y(G_b, C_m, \omega_2)}{i\cdot(\omega_1-\omega_2)}$$

The ADVantage System uses a constant current, so if the above dual frequency sample is taken at two different heart positions, a and b, they can be connected via the RMS current: $I^2 := I\cdot\overline{I}$

$V\cdot Y = I$ therefore $Y_a\cdot V_a\cdot\overline{Y_a}\cdot\overline{V_a} = Y_b\cdot V_b\cdot\overline{Y_b}\cdot\overline{V_b}$ or $\frac{V_a\cdot\overline{V_a}}{V_b\cdot\overline{V_b}} = \frac{Y_b\cdot\overline{Y_b}}{Y_a\cdot\overline{Y_a}}$

This relation can be solved for σ/ε in terms of the two Y's, the two C_m's, ω, and the two G_b's. Only the G_b's are unknown at this point.

$$M = \left[\begin{array}{l} \frac{C_{m\_a}\cdot G_{b\_a}\cdot Y_b\cdot\overline{Y_b} - C_{m\_b}\cdot G_{b\_b}\cdot Y_a\cdot\overline{Y_a}}{C_{m\_b}^2\cdot Y_a\cdot\overline{Y_a} - C_{m\_a}^2\cdot Y_b\cdot\overline{Y_b}} + \frac{\sqrt{-\omega^2\cdot\left[C_{m\_a}^4\cdot Y_b^2\cdot\left(\overline{Y_b}\right)^2 + C_{m\_b}^4\cdot Y_a^2\cdot\left(\overline{Y_a}\right)^2\right] + \overline{Y_b}\cdot\overline{Y_a}\cdot Y_a\cdot Y_b\cdot\left(2\cdot C_{m\_a}^2\cdot C_{m\_b}^2\cdot\omega^2 + C_{m\_a}^2\cdot G_{b\_b}^2 - 2\cdot C_{m\_a}\cdot C_{m\_b}\cdot G_{b\_a}\cdot G_{b\_b} + C_{m\_b}^2\cdot G_{b\_a}^2\right)}}{C_{m\_b}^2\cdot Y_a\cdot\overline{Y_a} - C_{m\_a}^2\cdot Y_b\cdot\overline{Y_b}} \\ \frac{C_{m\_a}\cdot G_{b\_a}\cdot Y_b\cdot\overline{Y_b} - C_{m\_b}\cdot G_{b\_b}\cdot Y_a\cdot\overline{Y_a}}{C_{m\_b}^2\cdot Y_a\cdot\overline{Y_a} - C_{m\_a}^2\cdot Y_b\cdot\overline{Y_b}} - \frac{\sqrt{-\omega^2\cdot\left[C_{m\_a}^4\cdot Y_b^2\cdot\left(\overline{Y_b}\right)^2 + C_{m\_b}^4\cdot Y_a^2\cdot\left(\overline{Y_a}\right)^2\right] + \overline{Y_b}\cdot\overline{Y_a}\cdot Y_a\cdot Y_b\cdot\left(2\cdot C_{m\_a}^2\cdot C_{m\_b}^2\cdot\omega^2 + C_{m\_a}^2\cdot G_{b\_b}^2 - 2\cdot C_{m\_a}\cdot C_{m\_b}\cdot G_{b\_a}\cdot G_{b\_b} + C_{m\_b}^2\cdot G_{b\_a}^2\right)}}{C_{m\_b}^2\cdot Y_a\cdot\overline{Y_a} - C_{m\_a}^2\cdot Y_b\cdot\overline{Y_b}} \end{array}\right]$$

Measuring Muscle Properties without a Probe Cont.

If we now subtract the admittance at the two heart positions, we get another expression for σ/ε where M is σ/ε.

$$M = -\frac{G_{b\_a} - G_{b\_b} - Y_a + Y_b}{C_{m\_a} - C_{m\_b}} - \omega \cdot i$$

Equating this last relationship with the previous one, eliminates M. We now have a expression with only the unknowns being the G_b's. The G_b's can be removed by once again considering the drive current. If the current for one frequency is I and this is produced be a drive voltage V, then since the magnitude of I is constant we can write:

$$I^2 = \left(G_{b\_a} + M \cdot C_{m\_a} + i \cdot \omega \cdot C_{m\_a}\right) V_a \cdot \left[\left(G_{b\_a} + M \cdot C_{m\_a} - i \cdot \omega \cdot C_{m\_a}\right) \overline{V_a}\right]$$

$$I^2 = V_b \cdot \left(G_{b\_b} + M \cdot C_{m\_b} + i \cdot \omega \cdot C_{m\_b}\right) \cdot \left[\overline{V_b} \cdot \left(G_{b\_b} + M \cdot C_{m\_b} - i \cdot \omega \cdot C_{m\_b}\right)\right]$$

These can be solved for the G_b's in terms of M and the measurable quantities I and V. This will reintroduce M into the relation while eliminating the G_b's. The resulting relation is rather large, but can be solved for M. After some manipulation the following relation obtains:

$$M\left(Y_a, Y_b, V_a, V_b, I, \omega, C_{m\_a}, C_{m\_b}\right) := \left[\frac{\sqrt{\overline{Y_b} \cdot \overline{Y_a} \cdot I^2 \cdot Y_a \cdot Y_b \cdot \left(\frac{C_{m\_a}^{\,2}}{V_b \cdot \overline{V_b}} + \frac{C_{m\_b}^{\,2}}{V_a \cdot \overline{V_a}}\right) - \frac{2 \cdot C_{m\_a} \cdot C_{m\_b} \cdot Y_a \cdot Y_b \cdot \overline{Y_a} \cdot \overline{Y_b} \cdot \sqrt{I^2 \cdot V_a \cdot \overline{V_a} - C_{m\_a}^{\,2} \cdot V_a^{\,2} \cdot \omega^2 \cdot \left(\overline{V_a}\right)^2} \cdot \sqrt{I^2 \cdot V_b \cdot \overline{V_b} - C_{m\_b}^{\,2} \cdot V_b^{\,2} \cdot \omega^2 \cdot \left(\overline{V_b}\right)^2}}{V_a \cdot V_b \cdot \overline{V_a} \cdot \overline{V_b}} - \omega^2 \cdot \left[C_{m\_a}^{\,4} \cdot Y_b^{\,2} \cdot \left(\overline{Y_b}\right)^2 + C_{m\_b}^{\,4} \cdot Y_a^{\,2} \cdot \left(\overline{Y_a}\right)^2\right]}}{2 \cdot C_{m\_b}^{\,2} \cdot Y_a \cdot \overline{Y_a} - 2 \cdot C_{m\_a}^{\,2} \cdot Y_b \cdot \overline{Y_b}} + \frac{\sqrt{I^2 \cdot V_a \cdot \overline{V_a} - C_{m\_a}^{\,2} \cdot V_a^{\,2} \cdot \omega^2 \cdot \left(\overline{V_a}\right)^2} \cdot \left(Y_b \cdot \overline{Y_b} \cdot C_{m\_a} \cdot C_{m\_b} - 2 \cdot Y_b \cdot \overline{Y_b} \cdot C_{m\_a}^{\,2} + Y_a \cdot \overline{Y_a} \cdot C_{m\_b}^{\,2}\right)}{2 \cdot V_a \cdot \overline{V_a} \cdot \left(C_{m\_b}^{\,2} \cdot Y_a \cdot \overline{Y_a} - C_{m\_a}^{\,2} \cdot Y_b \cdot \overline{Y_b}\right) \cdot \left(C_{m\_a} - C_{m\_b}\right)} + \frac{\sqrt{I^2 \cdot V_b \cdot \overline{V_b} - C_{m\_b}^{\,2} \cdot V_b^{\,2} \cdot \omega^2 \cdot \left(\overline{V_b}\right)^2} \cdot \left(Y_b \cdot \overline{Y_b} \cdot C_{m\_a}^{\,2} + Y_a \cdot \overline{Y_a} \cdot C_{m\_a} \cdot C_{m\_b} - 2 \cdot Y_a \cdot \overline{Y_a} \cdot C_{m\_b}^{\,2}\right)}{2 \cdot V_b \cdot \overline{V_b} \cdot \left(C_{m\_b}^{\,2} \cdot Y_a \cdot \overline{Y_a} - C_{m\_a}^{\,2} \cdot Y_b \cdot \overline{Y_b}\right) \cdot \left(C_{m\_a} - C_{m\_b}\right)} + \frac{Y_a - Y_b}{2 \cdot \left(C_{m\_a} - C_{m\_b}\right)} + \frac{\omega \cdot i}{2}\right]$$

This is an expression for σ/ε based only remotely observable parameters.

In conclusion, it would appear that it is possible to measure the properties of muscle using only the admittance catheter using two frequencies at two heart positions.

Proper PV Catheter Placement in the Left Ventricle

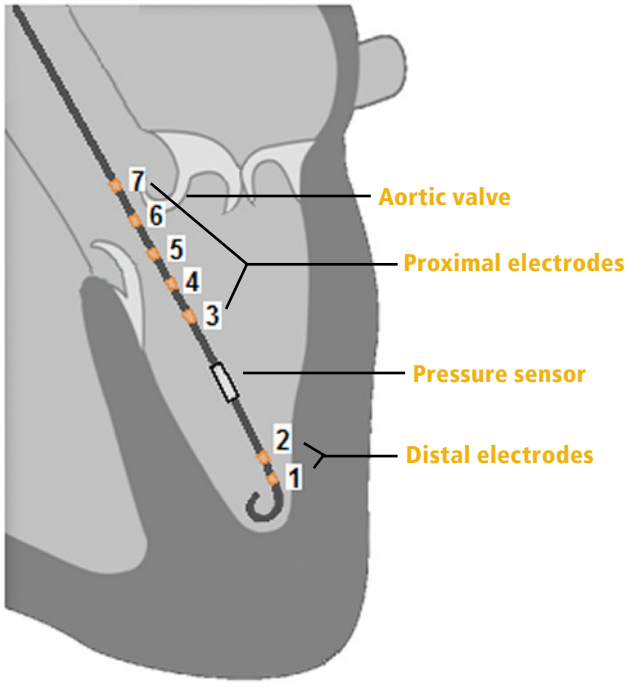
VIEWING THE PRESSURE VS. MAGNITUDE LOOPS AND PHASE SIGNAL DURING CATHETER POSITIONING IN THE VENTRICLE CAN ASSIST IN PROPER PLACEMENT.

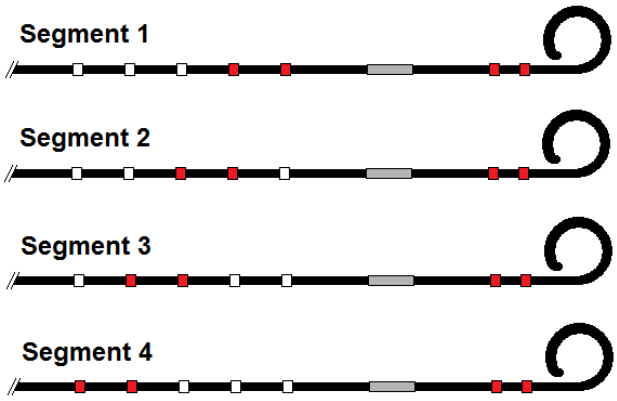
Correct placement of the Catheter in the ventricle is important for accurate data collection. Always use care when inserting the Catheter past the aortic valve as excessive force can cause Catheter damage, especially in small, rodent Catheters. Variable Segment Length (VSL) Catheters have four segment length settings. Select the longest segment length that gives physiological shaped pressure vs magnitude loops. The typical range of magnitude values for healthy animals with the specified body weights and stroke volumes are as shown:

SPECIES	BODY WEIGHT	STROKE VOLUME (SV)	MAGNITUDE |γ| VARIATION	PHASE (DEGREE)
Mouse	20 - 25 g	18 - 23 µL	≥ 200 µS	4 - 8
Rat	300 - 400 g	270 - 360 µL	≥ 500 µS	3 - 7
Pig	35 - 45 kg	30 - 40 ml	≥ 2.5 mS	1 - 5

If using a VSL Catheter, start with the shortest segment length (segment 1) for initial insertion.

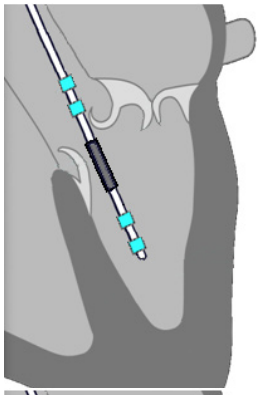
The phase signal allows you to visualize the proximity of the Catheter to the heart wall and can help in Catheter placement. The signal should be periodic in shape with a relatively low mean value (< 10°). Position the Catheter for the lowest mean phase signal (center of the LV). If the phase signal is excessively noisy, try repositioning the Catheter slightly or remove any sources of interference that may be attached to the animal.

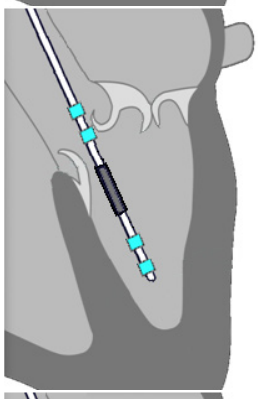


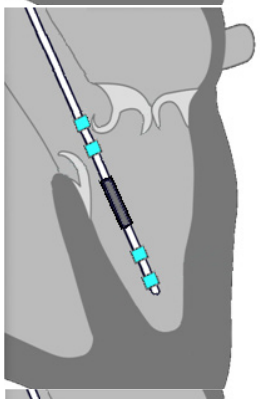


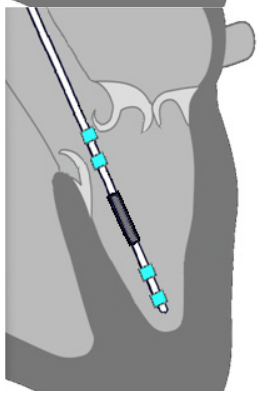
Four selectable segment lengths (active rings in red). Segment 1 is the shortest (for smaller hearts) while segment 4 is the longest (for larger hearts). The distal electrode pair (1 & 2) remain the same for all segments.

Proper PV Catheter Placement in the LV Cont.









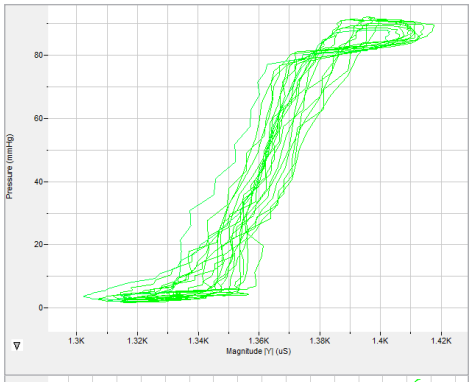
The evolution of pressure vs magnitude loops as the Catheter is inserted into the left ventricle from the aorta follows:

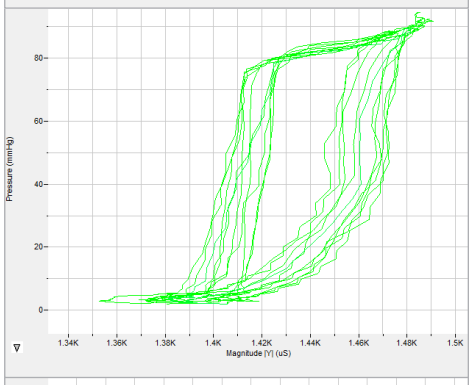
1. Pressure sensor is in the LV, just past the aortic valve. The two distal volume electrodes are in the LV. The two proximal electrodes are in the aorta.

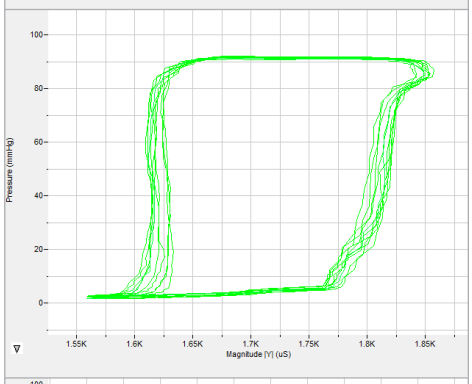
2. Pressure sensor is in the LV, just past the aortic valve. The two distal volume electrodes are in the LV. The two proximal electrodes are in the aorta, very close to the aortic valve.

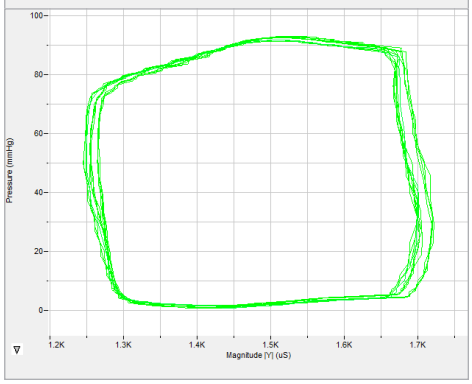
3. Pressure sensor is in the center of the LV. The two distal volume electrodes are in the LV. The two proximal electrodes are on either side of the aortic valve.
   - If the Catheter is too long for the ventricle size being studied, this may be the best position possible. If available, a smaller Catheter should be used instead.

4. Pressure sensor and volume rings are completely inside the LV. The most proximal electrode is situated very close to the aortic valve in the LV.
   - This is the ideal Catheter position.
   - Once the ideal depth has been determined, check the phase signal to ensure the Catheter is appropriately centered in the ventricle.









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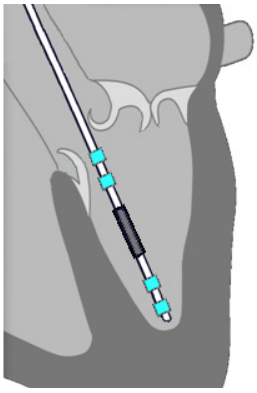
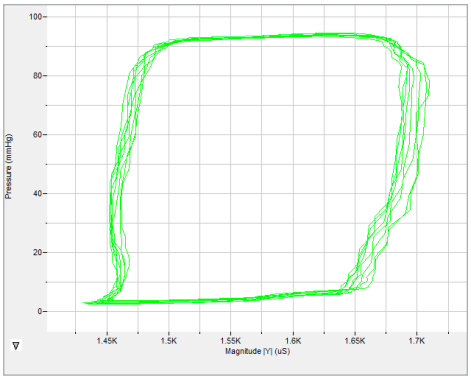
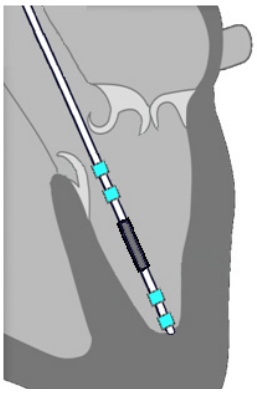
THE MEASURE OF BETTER RESULTS.

Proper PV Catheter Placement in the LV Cont.

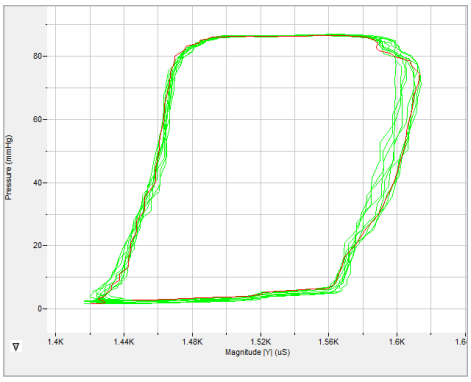


1. Catheter tip is very close to the apex. The distal electrodes almost touch the apex.
   - This Catheter position is acceptable. During IVC occlusions, the proximal electrodes could be pushed out of the LV into the aorta.

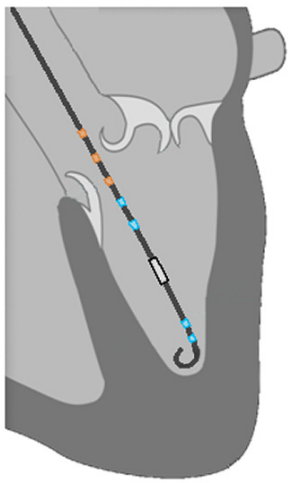




2. Catheter is jammed into the apex. The |Y| variation is low (≈ 100 µS)
   - Non-ideal position and must be avoided, if possible. This position causes premature ventricular contractions (PVC) in rats and large animals.

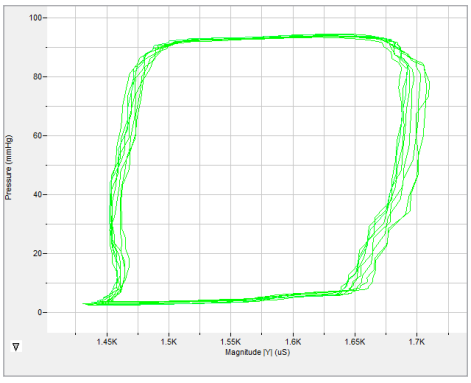


Once the Catheter is fully inserted, segment size can be adjusted on VSL Catheters. Increase the segment length one size at a time and observe the shape of the loops. Continue increasing the spacing until the shape of the loops no longer appears physiological. Then return to the previous segment. The following is an illustrative example of segment selection:

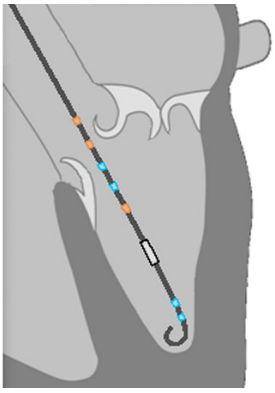


SEGMENT 1 ACTIVE ELECTRODES: 1, 2, 3, 4

- Selected segment is too short for the LV
- The shape of the Pressure vs. Magnitude loops look physiological
- Calculated volumes will be low due to missing volume at the base of the heart (near the valve)
- Select segment 2

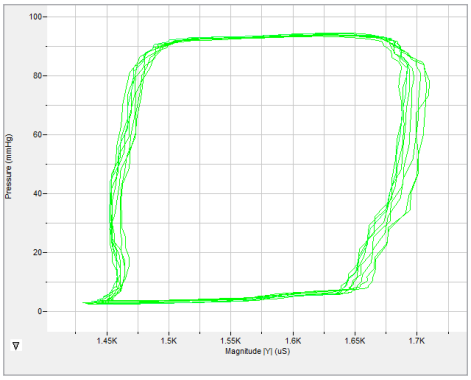


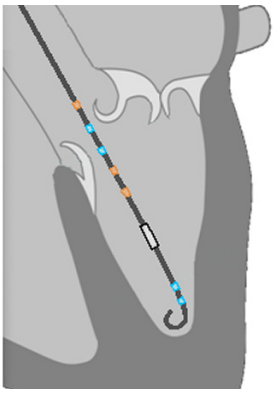
Proper PV Catheter Placement in the LV Cont.



SEGMENT 2 ACTIVE ELECTRODES: 1, 2, 4, 5

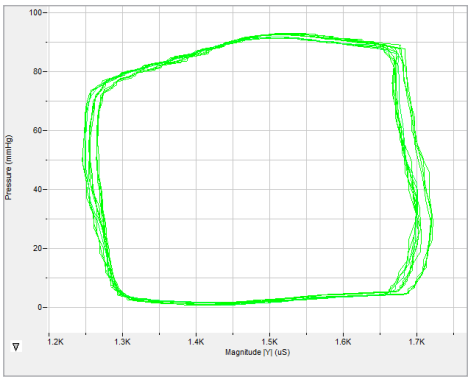
- Selected segment is too short for the LV
- The shape of the Pressure vs. Magnitude loops look physiological
- Calculated volumes will be low due to missing volume at the base of the heart (near the valve)
- Select segment 3

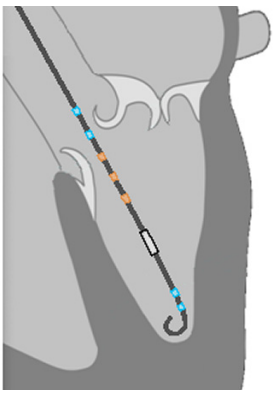




SEGMENT 3 ACTIVE ELECTRODES: 1, 2, 5, 6

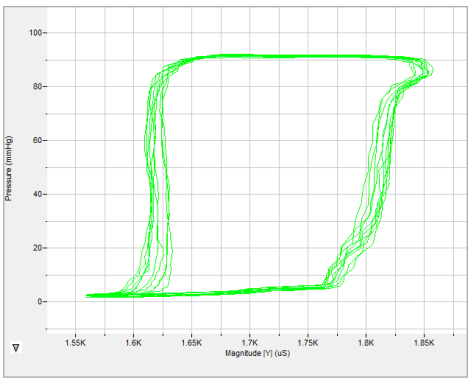
- Selected segment is the ideal length for the LV
- The shape of the Pressure vs. Magnitude loops look physiological
- Calculated volumes will be accurate
- Select segment 4





SEGMENT 4 ACTIVE ELECTRODES: 1, 2, 6, 7

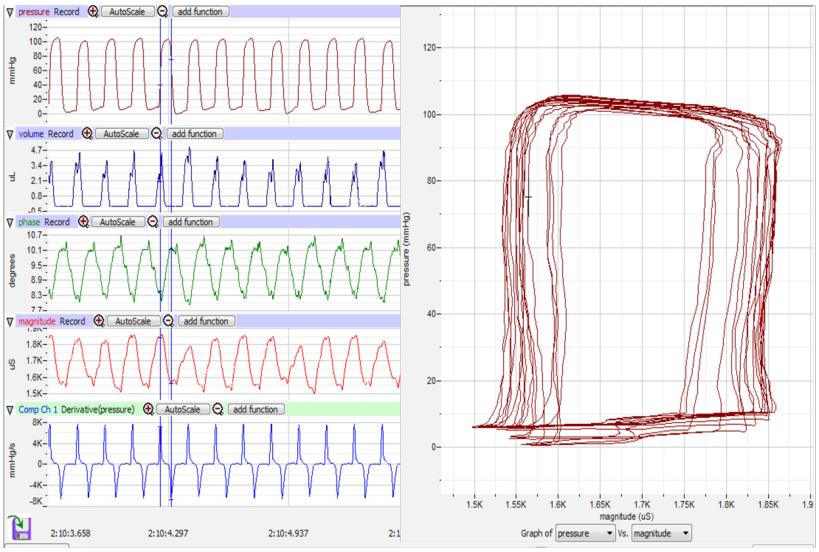
- Selected segment is too long for the LV
- The shape of the Pressure vs. Magnitude loops appears distorted
- Calculated volumes will be inaccurate
- Select segment 3 since it is the appropriate segment length for the LV



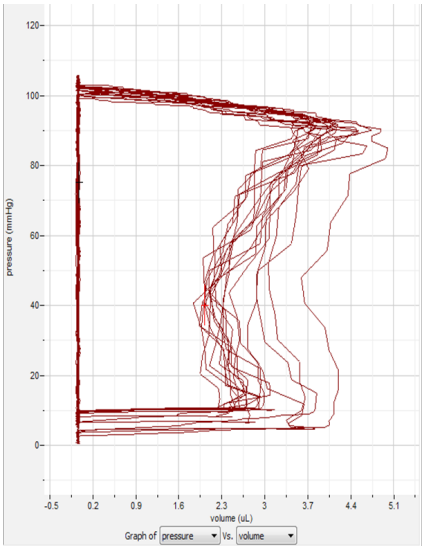
Proper PV Catheter Placement in the LV Cont.

Example of the importance of looking at phase signal.

In the first figure the pressure and magnitude data and loops look good. However, the phase range is too high for a mouse at ~8-10° which indicates that the catheter is not centered in the ventricle and should be repositioned. Switching the view to show the pressure-volume loops confirms that there is an issue with the Catheter position which is causing the volume to be calculated incorrectly.

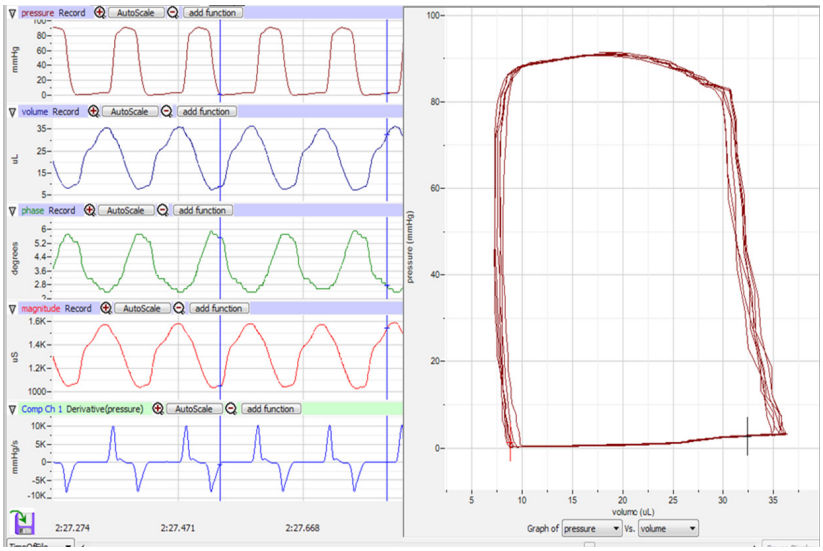


Mouse PV loop data and Pressure-Magnitude loops.



Pressure-Volume loops from the same data

Using the phase angle to guide the repositioning, the Catheter is adjusted and quality PV loops achieved. A baseline scan can now be performed and the user may move forward with the protocol.



Mouse PV loop data and Pressure-Volume loops after Catheter repositioning.

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Proper PV Catheter Placement in the Right Ventricle

Catheterization of the right ventricle (RV) is complex. Compared to the left ventricle (LV) the trajectory of PV Catheter passing into the RV depends not only on the site of its insertion but also on the complexity of RV chamber. For more information about the structure of the RV and it's associated PV signals, see "Right Ventricle PV Loops (RPV-11-tn)."

Invasive cardiac catheterization of the RV to collect pressure and volume remains the supreme measure to help to diagnose right ventricular hypertrophy in chronic pulmonary artery hypertension, post-MI remodeling changes of the RV, pulmonary or tricuspid valve diseases, and RV specific cardiomyopathies. It is also useful in determining prognosis and response to therapies.

In large animal models of RV research, placement of the PV Catheter is through the jugular or femoral vein (some investigators use branches of the pulmonary artery). For best results, guide the PV Catheter insertion using fluoroscopy and/or angiography. Once the Catheter is in the RV, phase signal can be used to help fine-tune positioning within the chamber.

RV stroke volume can be estimated, or measured using a Transonic Flowprobe positioned on the pulmonary artery. Collecting two lead ECG signals assists with data analysis by providing the timing of pulmonary or tricuspid valve closure(s).

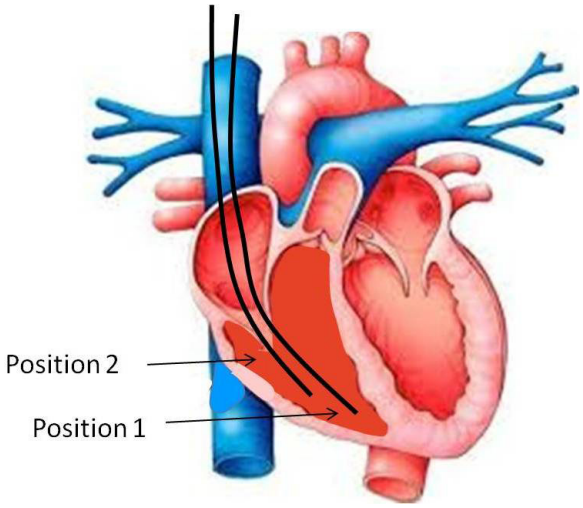
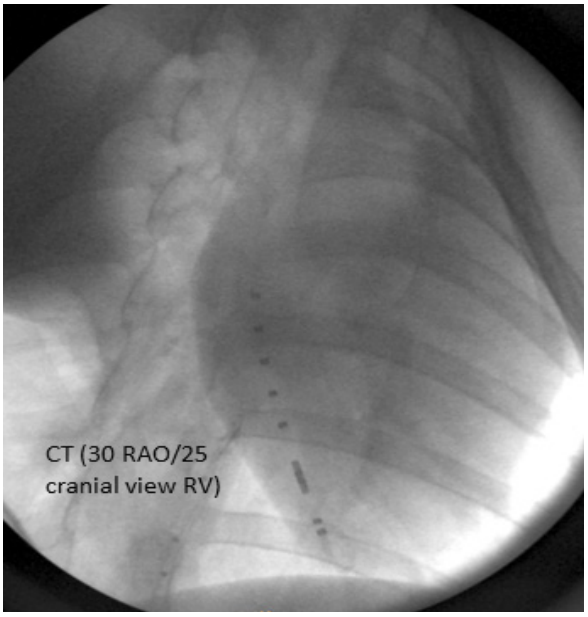


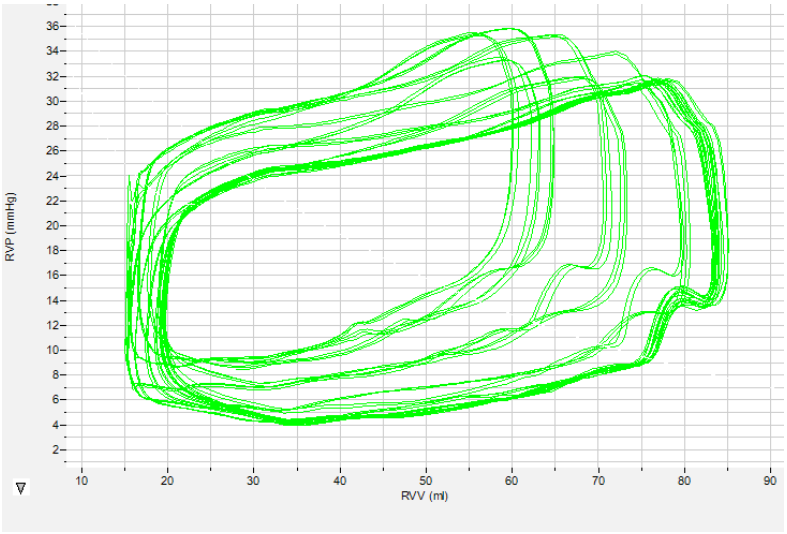
Fig. 1: Description of the PV Variable Segment Length (VSL) Catheter in the RV. Please note Position 1 is lower with the PV Catheter closer to the RV apex, as compared to position 2.

Data on the following two pages demonstrates RV PV measurement from 54 kg swine at 9 weeks post-pulmonary artery valve resection; modeling pulmonary insufficiency- induced RV volume overload.

Courtesy of Dr. Shelby Kutty, MD, FACC, Pediatric Cardiology, University of Nebraska Medical Center, Omaha, NE 68114, skutty@unmc.edu

Proper PV Catheter Placement in the RV Cont.





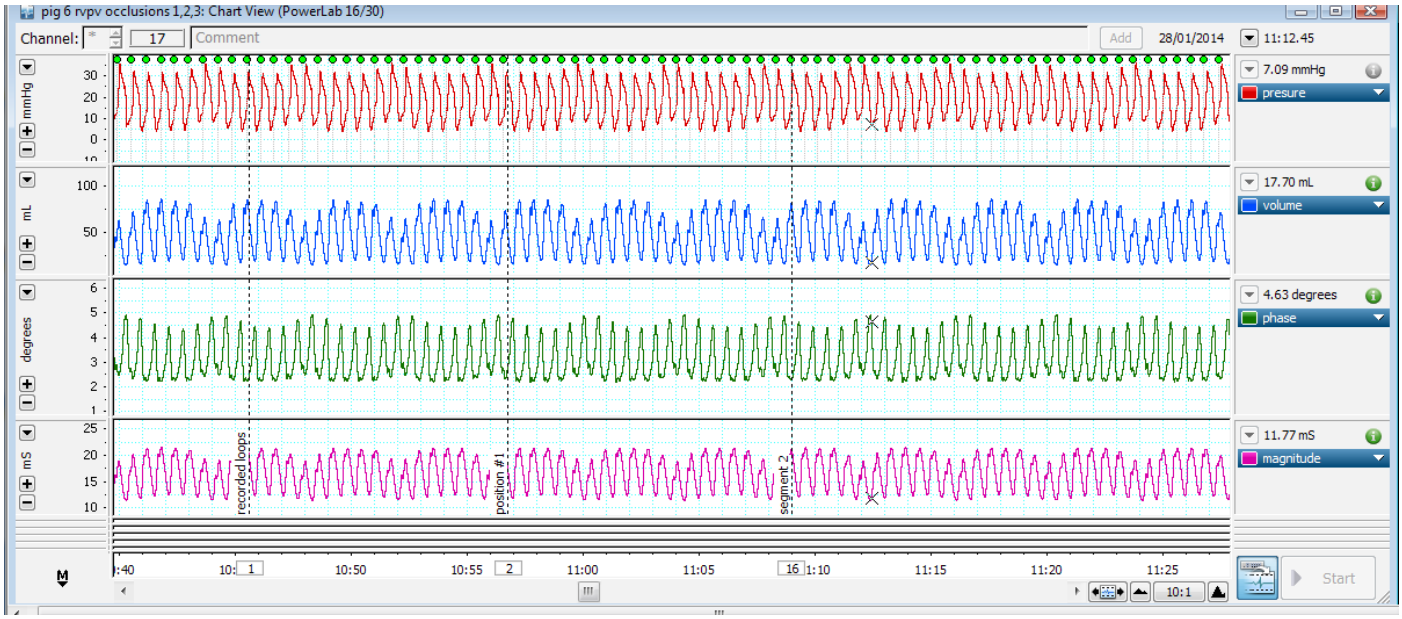
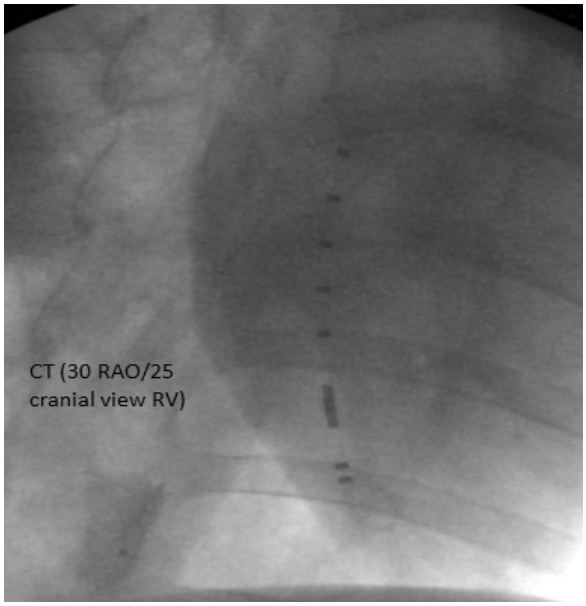
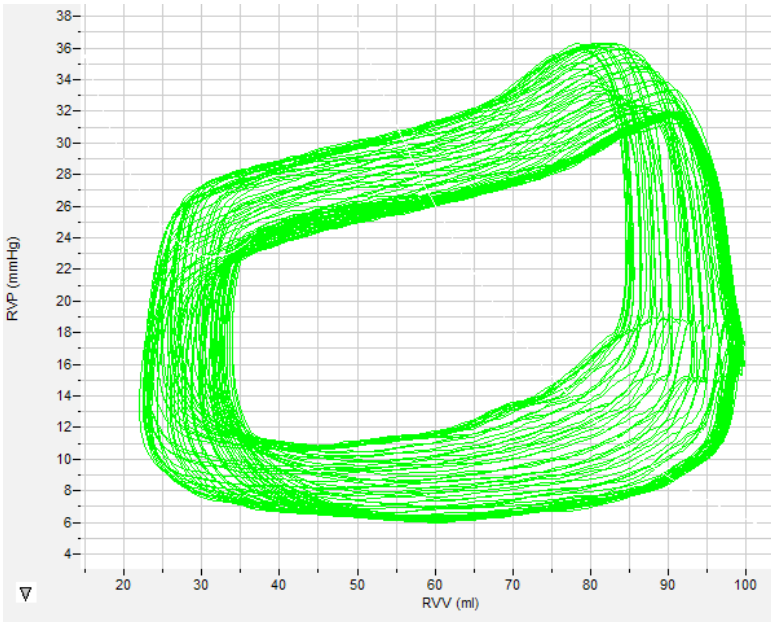


Fig 2 Position 1 with corresponding RV PV loops. The 7.0F PV VSL Catheter with selected segment number 2 is introduced in swine RV through jugular vein. Please note, the Phase signal (green trace) has a delta amplitude close to 2 degrees. Ideally, for the position centered in the RV it is best to find a low mean phase amplitude. Additionally, Magnitude (pink trace) should have an amplitude above 4 mS, with mean values ranging between 12-25 mS to achieve good RV PV loop tracing.

Proper PV Catheter Placement in the RV Cont.





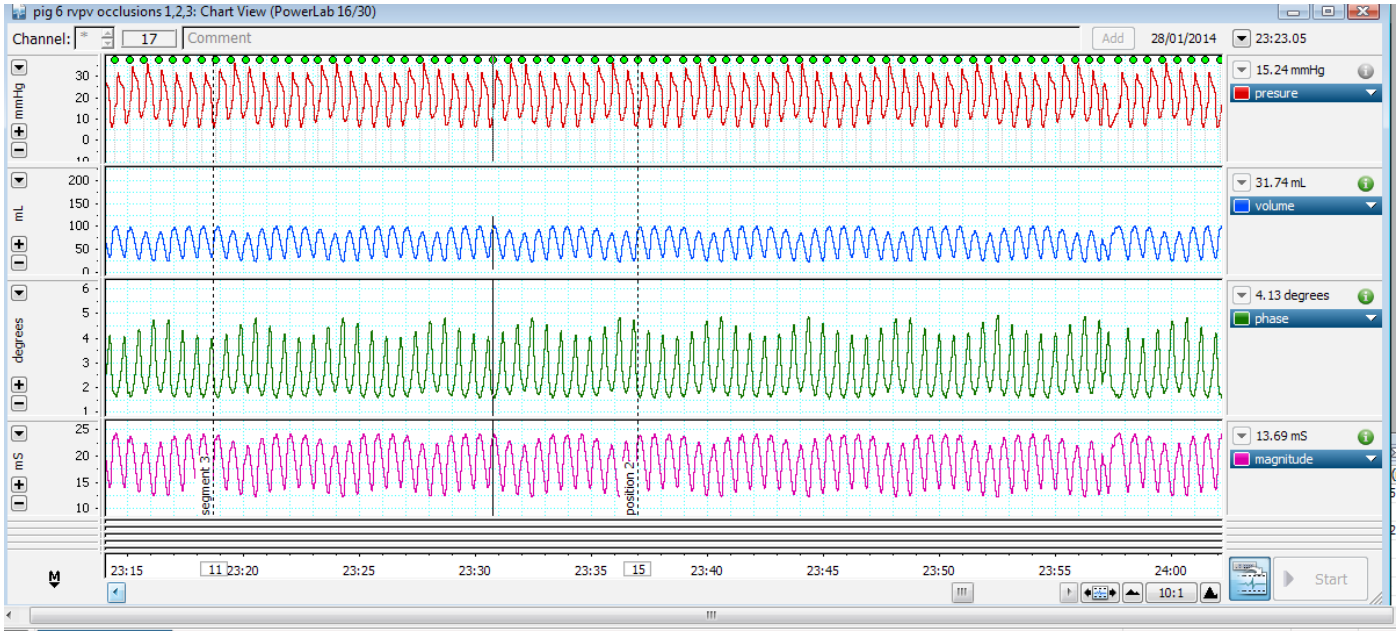


Fig 3: Position 2 with corresponding RV PV loops. The 7.0F PV VSL Catheter with selected segment number 3 is introduced in swine RV through jugular vein. Please note increase in delta Phase, now 2.75 (degrees), along with minimized mean reading indicating further PV Catheter position improvement in RV. Delta Magnitude signal improved along with its mean Magnitude that is now higher indicating better position in RV concurred with enhanced PV loop tracing.

Cleaning Guidelines for Scisense 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.

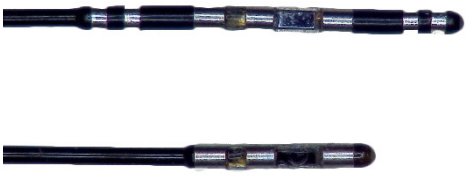


Fig. 2: Clean 1.2F Pressure and Pressure-Volume Catheters

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 below and ensure that no contamination remains through visual inspection.

Fig. 3: Clean 1.6F Pressure and 1.9F Pressure-Volume Catheters

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Cleaning Guidelines for Catheters Cont.

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.

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).

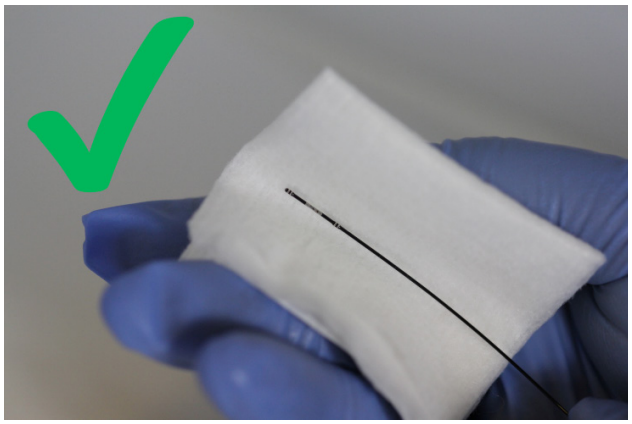


Fig. 4: Proper drying technique

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. 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).

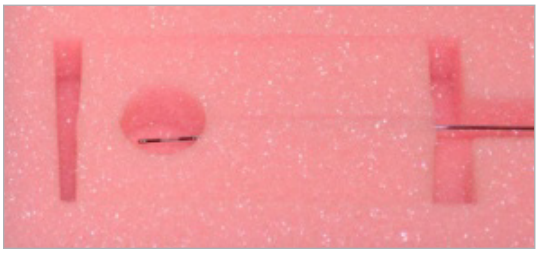


Fig. 6: Proper storage of catheter tip

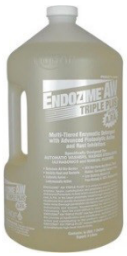
2. Disinfection Guide

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

Alternative Enzymatic Cleaning Agent:

- Tergazyme® - Enzyme-Active Powdered Detergent
  Whenever possible, use our suggested cleaning agent, Endozime. In the event that you do not have Endozime available in the lab, Tergazyme can be used if the directions are strictly followed – especially the rinsing protocol. Tergazyme includes a protease enzyme that removes proteinaceous soils, tissue, blood and body fluids. More information, including directions for use, can be found at:
  https://alconox.com/resources/standarddocuments/tb/techbull_tergazyme.pdf

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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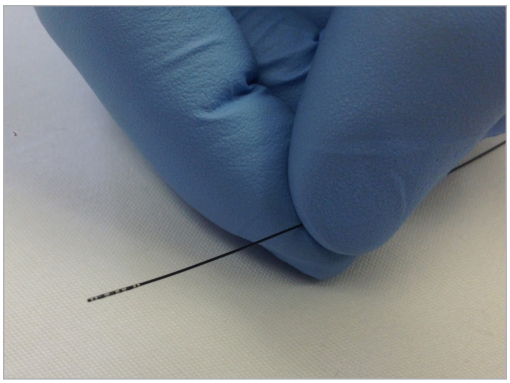
Optimizing Catheter Life Span

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.

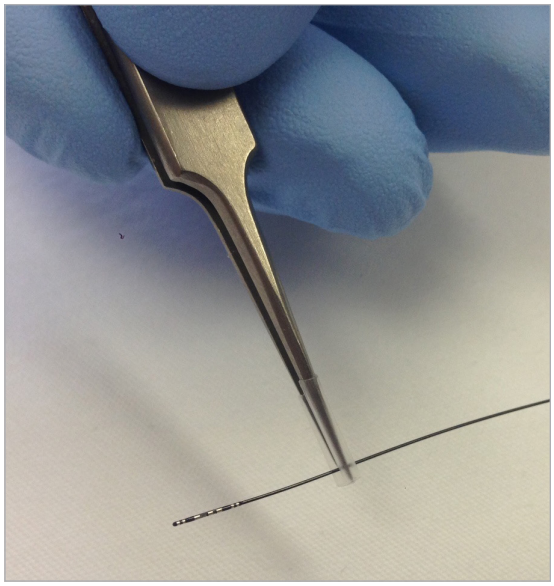


Correct catheter handling with fingers. Grip is along the shaft, well behind the sensitive catheter tip.

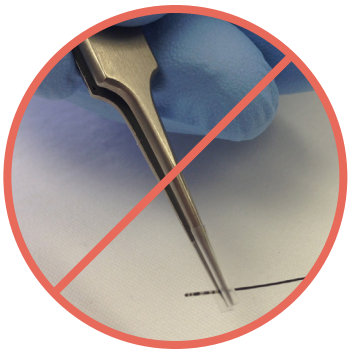
Place polyethylene tubing over the forceps' tips to protect the catheter body from damage.

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.

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.

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 dependant on your institution and country.

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

Rodent Anesthesia Guidelines Cont.

Cardiovascular Effects of Anesthetics

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.

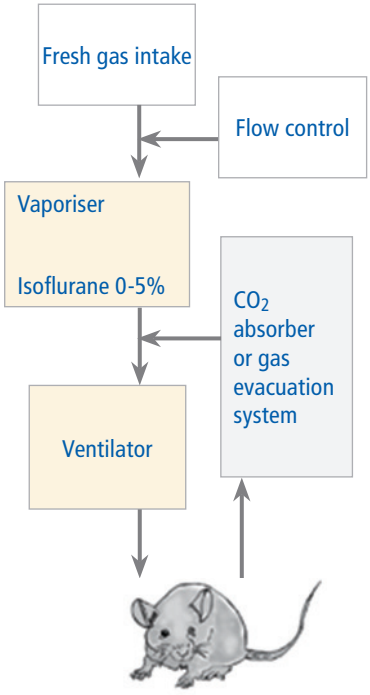
Semi-open System is commonly used today and includes all the Mapleson systems. This is typically used for animal anesthesia induction, usually a single branched system that uses a valve to control the pressure of the gas, and allows for waste gas to leave the system. This system can be further characterized 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.

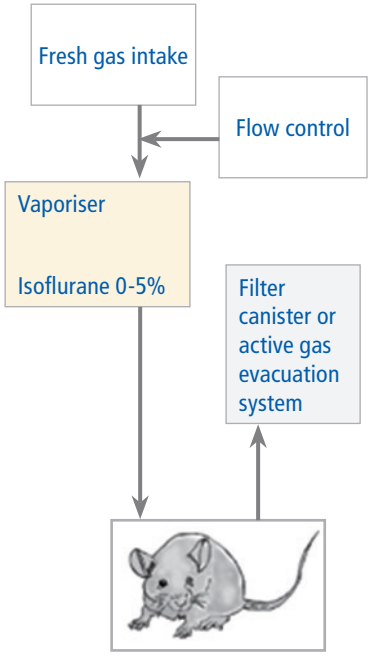
Breathing system components:

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

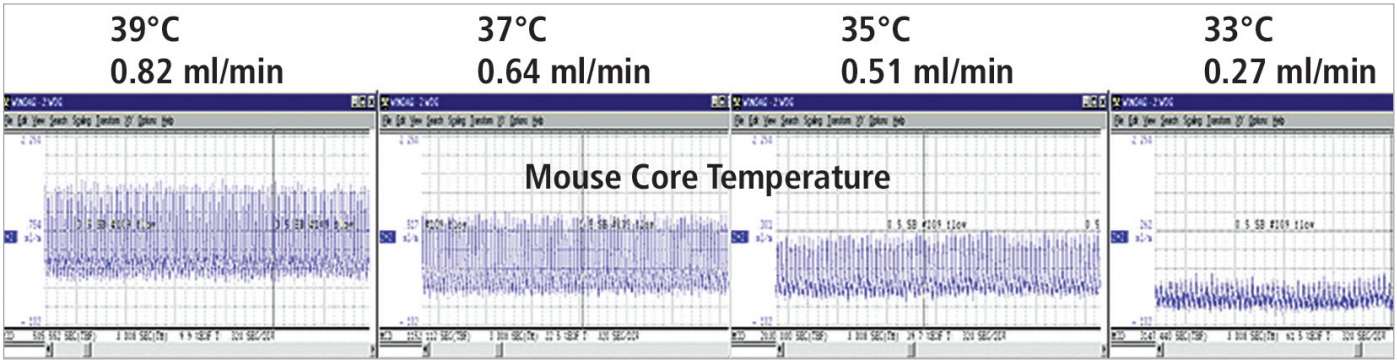
- When anesthetizing post-MI animals, maintenance of coronary artery pressure helps limit tachycardia.
- Induction of anesthesia can cause arrhythmias (junctional rhythms). Treat by reducing the dose of inhalation anesthetic or administering an anticholinergic.
- Halogenated volatile inhalation anesthetics (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.

SELECT REFERENCES

Alves HC, et. al. "Intraperitoneal propofol and propofol fentanyl, sufentanil and remifentanil combinations for mouse anaesthesia." Lab Anim. 2007 Jul; 41(3): 329-36 (PMID 17640460)

Blaudszun G, Morel DR. "Superiority of desflurane over sevoflurane and isoflurane in the presence of pressure-overload right ventricle hypertrophy in rats." Anesthesiology. 2012 Nov; 117(5): 1051-61 (PMID 22929732)

Gaertner, DJ, TM Hallman, FC Hankenson, MA Batchelder. 2008. Anesthesia and Analgesia in Rodents. Anesthesia and Analgesia in Laboratory Animals. Second Edition, Academic Press, CA.

Gargiulo S, et. al. "Mice Anesthesia, Analgesia, and Care, Part I: Anesthetic Considerations in Preclinical Research." ILAR J. 2012; 53(1): E55-69 (PMID 23382271)

Hildebrandt IJ, et. al. "Anesthesia and other considerations for in vivo imaging of small animals." ILAR J. 2008; 49(1): 17-26 (PMID 18172330)

Janssen BJ, et. al. "Effects of anesthetics on systemic hemodynamics in mice." Am J Physiol Heart Circ Physio. 2004 Oct; 287(4): H1618-24 (PMID 15155266)

Roth DM, et. al. " Impact of anesthesia on cardiac function during echocardiography in mice," Am J Physiol Heart Circ Physiol 2002; 282: H2134-40 (PMID 12003821)

Saha DC, et. al. "Comparison of cardiovascular effects of tiletamine-zolazepam, pentobarbital, and ketamine-xylazine in male rats." J Am Assoc Lab Anim Sci. 2007 Mar; 46(2): 74-80 (PMID 17343357)

Zeller A, et. al. "Mapping the contribution of β3-containing GABAA receptors to volatile and intravenous general anesthetic actions." BMC Pharm 2007; 7(2) (PMID 17319964)

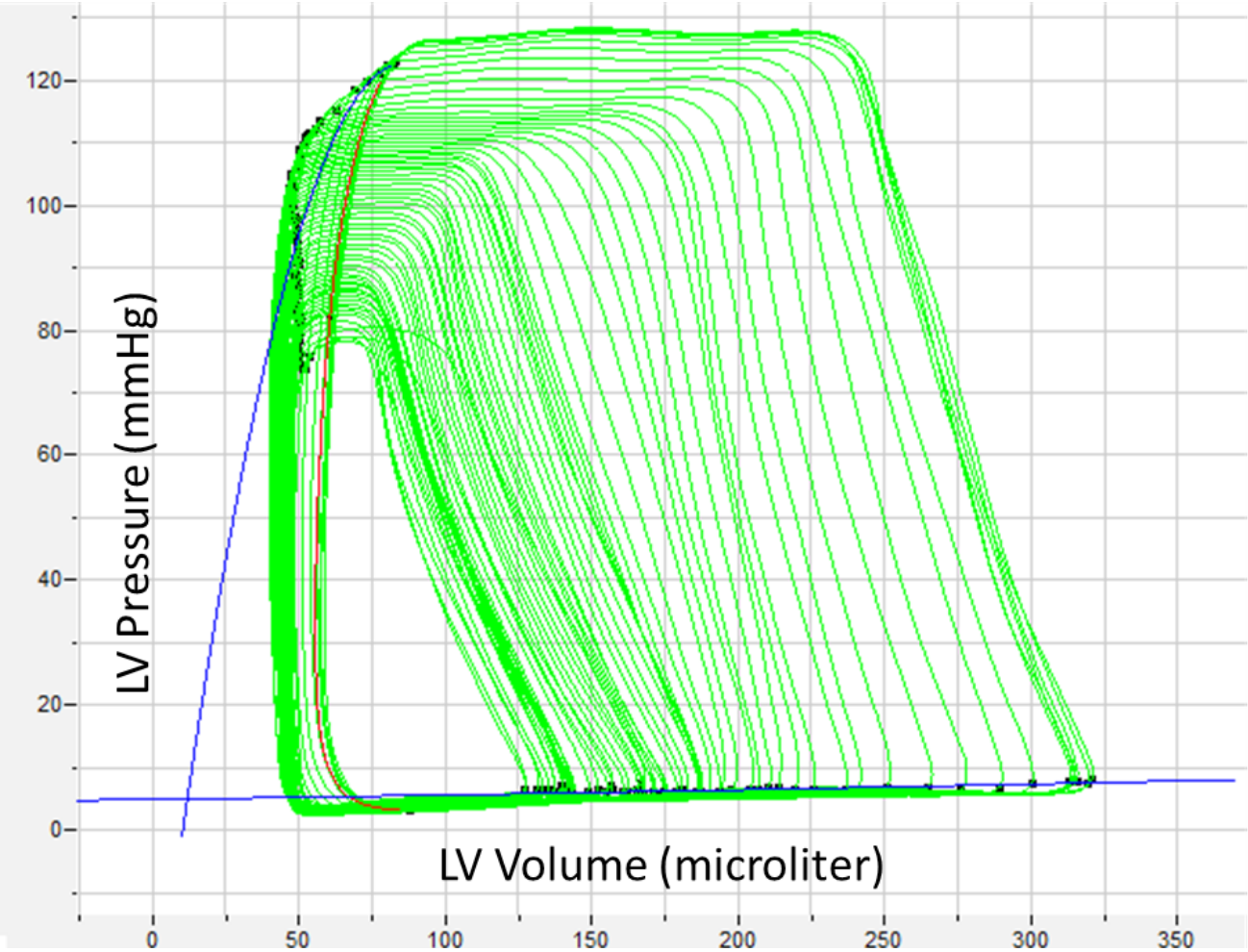
IDEAL ANESTHETIC AGENT

- 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.

Applications



Hemodynamics of Myocardial Infarction in Rodents

When using invasive methods to measure hemodynamic parameters in rodents, most researchers studying chronic myocardial infarct are using permanent suture-induced descending coronary artery occlusion. Usually, they perform the Pressure-Volume (PV) Catheter study at 4 weeks (28 days) post-MI (3, 4, 8). Some researchers extend the artery occlusion study time another 2 weeks (1, 2) to study the effect of chronic heart failure/animal survival.

Cardiac remodeling at 28 days after the onset of MI is characterized by the structural changes of the LV having impact on whole heart, such as infarcted regional-wall thinning, chamber dilatation, and hypertrophy in the viable region. The severity of these changes are based on position of occlusion (1, 3, 7). Signs of early post-infarction remodeling taking place in a mouse heart are described in the table below. Due to strain-dependency and genetic background of animals and also, with the position of coronary artery occlusion, the post-MI mortality varies. For more information on factors related to MI models including cellular and genetic influence please see "Translational Physiology of Myocardial Infarction" on page 73.

STAGE POST-MI	BEGINNING	DEVELOPMENT	TIME FRAME	CHANGES OF MYOCARDIUM	VENTRICULAR MECHANICS	VENTRICULAR FUNCTION
Coronary artery occlusion	Acute ischemia	Infarct enlargement	Minutes - hours	Disorder of structural proteins	Passive myocardium	Impaired systolic function
Infarct stiffening	Necrosis progression	Collagen formation	Hours - days	Edema, necrosis, and degradation	Increased stiffness and strength; infarct expansion	Impaired systolic function
Collagen formation	Fibrosis	Decreasing collagen formation	7 - 28 days	Increase in collagen content (scar formation)	Maximum stiffness	Impaired diastolic function
Decreased collagen formation	Remodelling	Scar thinning; the rest of myocardium hypertrophy	28 days +	Scar shrinkage and collagen cross-linking	Decrease in stiffness; scar anisotropy	Improved LV function

*Post-infarction stages in mouse based on work of Shioura et. al. (1)

When cardiac hemodynamics are measured by PV catheterization at 4 weeks post-MI, the load dependent parameters of cardiac function (e.g. SV, SW, CO, EF, dP/dt max/min, Tau) are reduced as compared to intact animals (1). At the same time, compensatory hypertrophy of surviving myocardium occurs roughly up until 6 weeks post-MI, after which decompensation occurs. LV decompensation is marked by a significant decrease of developed pressure, strikingly reduced SV, SW and CO and development of diastolic dysfunction. A noticeable negative outcome of diastolic dysfunction is seen in the rise in end-diastolic pressure (EDP). It is also common for the left atrial and pulmonary venous pressures to elevate leading to pulmonary congestion and edema.

COMPARING MAJOR SYSTOLIC AND DIASTOLIC LOAD INDEPENDENT INDICES (CONTROL VS. POST-MI)

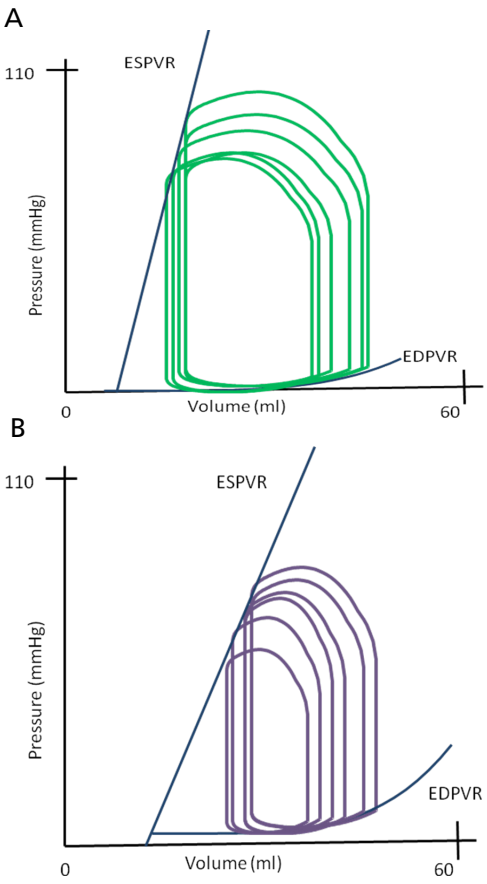
Systolic properties are characterized by the load-independent End Systolic Pressure-Volume Relationship (ESPVR) which is composed of the slope or end systolic elastance (Ees), and the volume axis intercept (V0). ESPVR can be characterized by either the quadratic or the linear equation. Generally, ESPVR is assumed to be influenced by afterload impedance (9), and when analyzed over wider ranges of contractile states, it was found to be non-linear (10) and the volume axis intercept is better estimated using quadratic rather than linear equation (11). For this reason when ESPVR (systolic functional contractility parameter) in rodents post-MI is compared to a control group, a simple t-test cannot be applied as it fails to account for covariance and statistical interdependence between Ees and V0. Therefore, it is best to report changes occurring in volume axis intercept (V0) and slope (Ees). To compare post-MI and control groups, analysis of covariance (ANCOVA) with dummy variable should be instituted (12). For further discussion of ESPVR comparison of groups see Burkhoff *et. al.* 2005 (13).

Hemodynamics of Myocardial Infarction Cont.

Diastolic properties are characterized by the load-independent End Diastolic Pressure-Volume Relationship (EDPVR). EDPVR is characterized by a non-linear curve fit of the change of ventricular pressure relative to change in ventricular volume (dP/dV). Post-MI changes characterized by an increase of collagen formation and scar cross-linking and shrinkage (see table) greatly influences final myocardial PV properties thus the position of the EDPVR . Slope of this relationship is called beta, also termed as chamber stiffness constant. When comparing EDPVR relationship post-MI one approach is to linearize it and use linear regression analysis with dummy variables or ANCOVA similar to ESPVR (12, 13).

As load independent parameters are measured, using pre-load reduction by temporary occlusion of inferior vena cava (IVC), a rightward shift of PV loops is observed. As a good internal control, it is imperative to select for analysis only those samples of IVC occlusion that were performed by similar technique including method of occlusion and vena cava location of preload reduction. Additionally, parameter such as ESPVR, EDPVR, time varying elastance (E_{max}), PRSW, dP/dt_{max} vs. EDV are all declining post-MI. PRSW (SW vs. EDV) deterioration reveals changes in systolic function independent of chamber geometry. Time-varying elastance indicates the LV chamber adjustments leading to decrease of compliance, defined by the proportionality between intraventricular pressure and volume. Left ventricular end-systolic elastance / effective arterial elastance increases at 4 weeks post MI indicating a worsening of this coupling ratio.

Using Admittance technique to assess load dependent and independent parameters in post-MI injured rodent heart has several distinct advantages over the traditional conductance method. There is no need for volume calibration of the catheter or hypertonic saline injection for parallel conductance determination with Admittance which saves time and reduces sources of error (6). By using Admittance method in rodent post-MI, an appropriate correction of the parallel conductance of injured cardiac muscle is achieved in real-time based on blood conductance calibrated to end systolic and end diastolic blood conductance and aortic flow. This occurs instantaneously while discarding the injured muscle parallel conductance (6). Admittance is also more insensitive to the impact of changes in heart geometry which may occur as a result of MI due to the ability to place the catheter in the center of the LV using Phase and Magnitude signals (5). See the PV Catheter Positioning Guide for placement methodology. Unguided conductance catheters can end up off-centered which gives inaccurate results (5).



Representative drawing of load-independent PV loops post-IVC occlusion at (A) beginning of study (control) and (B) at 4 weeks post-MI. At 4 weeks post-MI the purple PV loops shows characteristic rightward shift with decreased slope of ESPVR. ESPVR is progressively worsening and continues to decline with time following MI. LV chamber remodelling post-MI leads to increased stiffness with decreased preload capacity during diastole as seen in changes to the EDPVR. The rise of EDP at 4 weeks post-MI leads to increased effort of LV muscle against which heart has to work during the filling phase. Additionally, rodents with healed infarcts operate at higher EDV at 6-10 weeks MI post as compared to healthy hearts.

Hemodynamics of Myocardial Infarction Cont.

REFERENCES

(1) Shioura KM, Geenen DL, Goldspink PH. "Assessment of cardiac function with the pressure-volume conductance system following myocardial infarction in mice." Am J Physiol Heart Circ Physiol 293: H2870–H2877, 2007.

(2) Patten RD, Aronovitz MJ, Deras-Mejia L, Pandian NG, Hanak GG, Smith JJ, Mendelsohn ME, Konstam MA. "Ventricular remodeling in a mouse model of myocardial infarction." Am J Physiol. 1998; 274:H1812–1820.

(3) Takagawa J, Zhang Y, Wong ML, Sievers RE, Kapasi NK, Wang Y, Yeghiazarians Y, Lee RJ, Grossman W, Springer ML. "Myocardial infarct size measurement in the mouse chronic infarction model: comparison of area- and length-based approaches." J Appl Physiol. 2007 Jun;102(6):2104-11.

(4) Chen J, Petrov A, Yaniz-Galende E, Liang L, de Haas HJ, Narula J, Hajjar RJ. "The impact of pressure overload on coronary vascular changes following myocardial infarction in rats." Am J Physiol Heart Circ Physiol. 2013 Mar 1;304(5):H719-28.

(5) Clark JE, Marber MS. "Advancements in pressure-volume catheter technology stress remodelling after infarction." Exp Physiol. 2013 Mar;98(3):614-21.

(6) Clark JE, Kottam A, Motterlini R, Marber MS. "Measuring left ventricular function in the normal, infarcted and CORM-3-preconditioned mouse heart using complex admittance-derived pressure volume loops." J Pharmacol Toxicol Methods. 2009 Mar-Apr;59(2):94-9.

(7) Ahn D, Cheng L, Moon C, Spurgeon H, Lakatta EG, Talan MI. "Induction of myocardial infarcts of a predictable size and location by branch pattern probability-assisted coronary ligation in C57BL/6 mice." Am J Physiol Heart Circ Physiol. 2004 Mar;286(3):H1201-7.

(8) van den Borne SW, van de Schans VA, Strzelecka AE, Vervoort-Peters HT, Lijnen PM, Cleutjens JP, Smits JF, Daemen MJ, Janssen BJ, Blankesteijn WM. "Mouse strain determines the outcome of wound healing after myocardial infarction." Cardiovasc Res. 2009 Nov 1;84(2):273-82.

(9) Burkhoff D, De Tombe PP, Hunter WC. "Impact of ejection on magnitude and time course of ventricular pressure-generating capacity." Am J Physiol. 1993 Sep;265(3 Pt 2):H899-909.

(10) Sato T, Shishido T, Kawada T, Miyano H, Miyashita H, Inagaki M, Sugimachi M, Sunagawa K. "ESPVR of in situ rat left ventricle shows contractility-dependent curvilinearity." Am J Physiol. 1998 May;274(5 Pt 2):H1429-34.

(11) Claessens TE, Georgakopoulos D, Afanasyeva M, Vermeersch SJ, Millar HD, Stergiopulos N, Westerhof N, Verdonck PR, Segers P. "Nonlinear isochrones in murine left ventricular pressure-volume loops: how well does the time-varying elastance concept hold?" Am J Physiol Heart Circ Physiol. 2006 Apr;290(4):H1474-83.

(12) Slinker BK, Glantz SA. "Multiple regression for physiological data analysis: the problem of multicollinearity." Am J Physiol. 1985 Jul;249(1 Pt 2):R1-12.

(13) Burkhoff D, Mirsky I, Suga H. "Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers." Am J Physiol Heart Circ Physiol. 2005 Aug;289(2):H501-12.

Muscle Properties of Injured Murine Myocardium

After heart muscle is injured the properties of the muscle change due to remodeling of the injury and surrounding areas of myocardium, leading to a multifaceted myocardial tissue injury. After the onset of myocardial infarct (MI), pathological changes occur as early as 20 min post ischemia (1). It usually takes 2-4 hours to identify tissue necrosis, based on terminal blood supply, the sensitivity of cardiac myocytes to ischemia, individual cell's demand for nutrients and oxygen, and cardiac pre-conditioning. Healing takes at least 5-6 weeks (2). Healed myocardium that turns into sparse or entirely non-myocyte area becomes mostly resistive to the flow of current. These resistive areas contain collagen, elastin and other fibers, lacking cardiomyocytes with fully developed sarcolemas.

Murine myocardial resistivity is measured by a surface probe creating a sufficient electrical field to overcome anisotropy of the myocardial fibers. The majority of the current flows through the sarcolema as it has a lower impedance than the other tissue elements. The magnitude of impedance of the sarcolema is higher at lower frequencies. As the frequency is increased, the magnitude of impedance to flow of current decreases, effectively allowing more current flow through healthy tissue sarcolema. Given the current moving through sarcolema of healthy myocardium, in case of scar tissue composed from collagen and other fibrous materials, lacking presence of sarcolemas, current flow is limited. In case of scar tissue there should be no change in impedance even during increasing frequencies. The myocardial frequency-resistivity relationship varies, therefore resistivity must be defined for every animal group studied (e.g. genetically altered mice). In that respect low inter-animal variability exists thus usually 4-5 animals have to be measured to account for inter-strain, genetic variability (3). As the frequency of input signal is increased myocardial resistivity falls in a manner unique to the heart muscle. Temperature of the heart also has to be maintained as the resistivity of myocardium increases with lower temperatures.

Furthermore resistivity varies during the cardiac cycle due to the greater presence of conductive blood within the chambers and blood vessels and capillaries especially during the filling phase (late diastole). As blood is less resistive than muscle (i.e. blood is more conductive), an increased amount of blood in the electrical field effectively lowers the overall myocardial resistivity, contributing to intra-measurement variability. To minimize the contributions of blood, high vascular areas should be avoided if possible. Additionally, due to myocardial tissue anisotropy represented by cardiomyocytes orientation from endocardium towards the epicardium, surface probe orientation has to be adjusted to enable better current passage (3). It is therefore necessary to re-orient and measure several areas on the chamber's free wall to determine the mean value (3). A practical way to avoid tissue anisotropy to establish baseline values in healthy myocardium is to increase the depth of electrical field penetration, which would limit the impact of native syntitial orientation. Furthermore on cellular level, changes of composition of sarcolemal membrane within different injury or metabolic models alters overall properties of the myocardium. As certain metabolites, which are at this time incorporated into the remodeling myocardium e.g. free-fatty acids or glycogen, are current insulators, resistivity of tissue increases, successfully limiting augmentation of electrical field penetration capacity (3).

PERMITTIVITY/CONDUCTIVITY RATIO (σ/ε)

The recommended permittivity/conductivity ratio (σ/ε) for murine myocardium of 800,000 is based on actual measurements and has been validated in numerous research labs (4-6). Empirically tested surface probe depth penetration to measure healthy LV myocardium using fixed electrode spacing resulted in a penetration depth of approximately 0.6 mm (7). It would be speculative to assume without measurement that in cases of myocardial infarction, when the remodeling occurs and final scar settles in, that portions of the electric field might or might not penetrate beyond the infarcted myocardium into semi-conductive environment of the chamber blood pool.

Muscle Properties of Injured Murine Myocardium Cont.

PERMITTIVITY/CONDUCTIVITY RATIO (σ/ε) CONT.

It is recommended to use a surface probe in these cases to obtain measurements as damage on myocardial LV free wall leading to scarring can cover up to 43% of the surface area (9), based on the position of the coronary artery ligation (8) or mouse strain (10). It has been recommended that default values of 900,000 would accommodate changes occurring post-infarction and 700,000 for post-hypertrophied myocardial changes. These are based on the best information currently available. However, empirical muscle properties determinations are warranted in cases of genetically modified animals where the myocardial anisotropy could be different right from the beginning of an experiment in addition to post-injury. With this in mind, animals during concurrent metabolic consignments that are exposed to myocardial damage should also be measured. Furthermore, in cases of myocardial hypertrophy (recommended value for hypertrophy is 700,000) the LV myocardial permittivity and conductivity ratios of control and aortic banded mice as measured by the surface probe demonstrated that although the conductivity did not change significantly, the permittivity did (11,844 to 21,267 control to aortic banded mice, respectively) (5).

REFERENCES

(1) Jennings RB, Ganote CE. Structural changes in myocardium during acute ischemia. Circ Res. 1974 Sep; 35 Suppl 3:156-72.

(2) White H, et. al. Universal MI definition update for cardiovascular disease. Curr Cardiol Rep. 2014 Jun;16(6):492.

(3) Reyes M, et. al. Impact of physiological variables and genetic background on myocardial frequency-resistivity relations in the intact beating murine heart. Am J Physiol Heart Circ Physiol. 2006 Oct;291(4):H1659-69.

(4) van Hout GP, et. al. Admittance-based pressure-volume loops versus gold standard cardiac magnetic resonance imaging in a porcine model of myocardial infarction. Physiol Rep. 2014 Apr 22;2(4):e00287.

(5) Porterfield JE, et. al. Dynamic correction for parallel conductance, GP, and gain factor, alpha, in invasive murine left ventricular volume measurements. J Appl Physiol (1985). 2009 Dec;107(6):1693-703.

(6) Kutty S, et. al. Validation of admittance computed left ventricular volumes against real-time three-dimensional echocardiography in the porcine heart. Exp Physiol. 2013 Jun;98(6):1092-101.

(7) Raghavan K, et. al.Electrical conductivity and permittivity of murine myocardium. IEEE Trans Biomed Eng. 2009 Aug;56(8):2044-53.

(8) Takagawa J, et. al. Myocardial infarct size measurement in the mouse chronic infarction model: comparison of area- and length-based approaches. J Appl Physiol (1985). 2007 Jun;102(6):2104-11.

(9) Gao E, Koch WJ. A novel and efficient model of coronary artery ligation in the mouse. Methods Mol Biol. 2013;1037:299-311.

(10) van den Borne SW, et. al. Mouse strain determines the outcome of wound healing after myocardial infarction. Cardiovasc Res. 2009 Nov 1;84(2):273-82.

Translational Physiology of Myocardial Infarction

The main aspect of post-myocardial infarct/infarction (MI) research is to mimic conditions that lead to the development of myocardial ischemia. MI indicates irreversible myocardial injury resulting in necrosis of a significant portion of myocardium. Human clinical MI may be either of the non-reperfusion type, if the obstruction to blood flow is permanent, or the reperfusion type, if the obstruction is reversed after myocardial cell death. The infarct-affected areas are limited by distribution of the occluded vessel(s). In humans, the left main coronary artery occlusion generally results in a large antero-lateral and septum infarct, whereas occlusion of the left anterior descending coronary artery causes necrosis limited to the anterior wall. Hospital post-mortem findings in most cases reveal an acute thrombus overlying an atherosclerotic plaque in the coronary arteries. In these examples of vascular distribution, recreating this instance in animal research models has proved challenging due to the limitations caused by the fundamental difference between human and rodent cardiovascular physiology, carotid artery disease (CAD), and cellular and genetic makeup. Additionally, in the human population there is a known genetic polymorphism involving pathways of lipids, coagulation, and the renin-angiotensin system which does not currently have a known rodent analog.

CREATION OF RODENT MI MODEL

The natural development of MI stems from atherosclerosis (athero-gruel/sclerosis-hardening) plaque growth which occludes blood flow or suddenly ruptures. There is, however, difficulty in re-creating plaque growth in animal models which consistently leads to MI. Several diet supplementation methods have been used to simulate this natural disease progression. First, feeding cholesterol-rich diet can lead to chronic narrowing of coronary arteries and hypercholesterolemia, making them vulnerable to plaque buildup and thrombosis (1). Additionally, using mice lacking apoE-/- and LDLr-/- in combination with a high fat diet accelerates disease progression (2). While very similar to the natural mechanism, these methods are difficult to replicate due to the inherent variability of plaque behavior. One of the complexities to studying vulnerable plaques is that they do not produce a significant stenosis before they rupture and cause an acute MI. Additionally, some of the vulnerable plaques are short-lived and might resolve spontaneously. This plaque behavior makes its development hard to detect or track. Finally, it can be very complex to control the location or extent of MI when it occurs. Due to these difficulties with animal model disease-like-state recreation, interventional (surgery) models are often used.

There are two main interventional methods of MI induction in rodents, vascular occlusion and cryoinjury. Vascular occlusion can be used to either fully, partially or temporary restrict blood flow though a coronary artery. In rodents there are temporary occluders such as hanging weights (3), and different methods of permanent or temporary suture ligation (1, 4). There are also other methods for occlusion of coronary arteries mostly in large animals including: occluders (U-shaped, ring-shaped, ameroid constrictors), balloon inflation in coronary artery.

Currently the preferred method of introduction of MI in rodents is permanent suture ligation of the left descending coronary artery. The advantage of using permanent occlusion is that it mimics the behavior of a complete plaque blockage by inducing hypoxia and ischemia. It is important to note that rodent coronary arteries have different locations and branching patterns as compared to human coronary arteries (5, 6, and 7). For example, rats do not have the circumflex artery (8), therefore, the proximal region of the left coronary artery gives origin to the septal branch and to the branch that corresponds to the circumflex artery (9).

An alternative method to occlusion is creation of infarction by freeze-thaw injury (10) or cryoinjury (11, 12). This method allows for a specific region of tissue to be damaged unconstrained of coronary artery physiology. While this method has good reproducibility, it is less physiologically relevant as it causes tissue injury by different mechanism(s). This in turn impacts the response to infarct as seen by the delayed remodeling as compared to occlusion induced infarcts (12). Since a capable trained rodent surgeon is able to create permanent suture ligations with high reproducibility, using freeze-thaw or cryoinjury methods as MI induction model requires careful consideration for its translation and should be subjected to careful scrutiny before the project begins.

Translational Physiology of Myocardial Infarct Cont.

A common downside to artificially induced rodent myocardial infarcts is its relative low-emergence of other clinical symptoms associated with heart failure (pulmonary congestion, chest tightness, dyspnea, cachexia, ST-elevation myocardial infarction (STEMI) and non ST-elevation infarction (non-STEMI)). Additionally, there are a lack of rodent observational studies that pay specific attention to morbidity and mortality post-MI resulting from arrhythmias, cardiac rupture, heart failure, valve insufficiency, and embolization. As these comorbidities have the potential to alter the type, timing or extent of physiological responses, many rodent models are inherently limited in their clinical pathophysiology-translational bench-to bedside value.

CELLULAR AND GENETIC INFLUENCES

The heart's response to stresses such as MI is influenced, in part, by cellular phenotypic composition and genetics. For example, an adult mouse heart consists of approximately 45% non-cardiomyocytes and 55% cardiomyocytes, whereas adult rat and human hearts consists of around 70% of non-cardiomyocytes and 30% of cardiomyocytes (13). More studies are necessary to better characterize the two major cell types and their roles, including their intercellular interactions, during and post-MI. Without this knowledge it is very challenging to determine if and, to what extent heart, cellular composition affects cardiac behavior.

Genetic variations within species also factor into cardiovascular behavior as seen by strain-dependent difference in mortality rates. In a study by Liu *et. al.* the mortality of Sprague-Dawley rats was 36%, whereas in Lewis inbred rats it was significantly lower at 16% (14). Mice mortality associated with MI induction ranges from 37–50% (15). Furthermore, when different genetic backgrounds of mice were studied post-MI the highest incidence of post-MI infarct rupture, which typically occurred at 3–6 days post-MI, was in 129S6 mice (62%), followed by C57Bl6 (36%), FVB (29%), Swiss (23%), and BalbC (5%) (16). This incidence of high infarct rupture in 129S6 strain mice was associated with highest systolic blood pressure and presence of inflammation in the area. It must then be determined if those responses (high BP and inflammation) are physiologically accurate. Once the importance and relevance of genetically controlled comorbities are determined, they aid in choosing an appropriate translational model.

DISEASE RESPONSE BEHAVIOR

Equally important in mimicing a post-MI condition is having a meaningful biological response. In case of arrhythmias originating from post myocardial injury, there are differences between rodents and humans due to the dissimilar locations of major cardiac electrical axis. Different apoptosis progression, heart rate, and activity of various ionic channels also play a role in arrhythmias (17). However, the rat animal model can be ideal as compared to human in the assessment of various therapeutic interventions addressing arrhythmias due to a high frequency of ischemic ventricular arrhythmias in a repetitive, self-terminating manner (18).

Myocardial infarct size and LV chamber dilation are more pronounced in experimental rodent model systems as compared to human infarct. This adds an additional level of complexity to comparing infarct severity between species. According to the Killip-Kimball classification of infarct size on LV dysfunction, Class I is for small infarcts while Class IV is the most severe (the majority being fatal) with major necrosis involving more than 30% of the LV free wall. Similar pre-clinical classification of infarct size or method of correlating rodent infarct to the Killip-Kimball system is missing in animal models. This important classification can be incorporated into rodent models given the fact that rodent infarcts usually involve a much larger percentage of the cardiac tissue. At 21 days post-MI, rats with small MI (i.e. 4-30%) have no apparent impairment in baseline hemodynamics or peak indices of pumping and pressure generating ability when compared to the sham non-MI rats. Moderate MI between (31- 46%) is associated with normal baseline hemodynamics, but reduced peak flow indices and developed pressure. Large MI (46% and more) present congestive heart failure, with elevated filling pressures, reduced CO, and a minimal capacity to respond to pre- and afterload stresses (19). Note: In rodents the same percentage of infarct size (30%) which corresponds to severe in humans is classified as small, with minimal long term effects.

Translational Physiology of Myocardial Infarct Cont.

Remodeling behavior in rodents has some distinct differences as compared to humans. Post-MI cardiac remodelling in rodents is characterized by biventricular remodelling such that myocytes from the LV, RV and intra-ventricular septum are elongated to about the same extent, and thickness of septum increases (20). In humans, remodeling is centralized to the damaged ventricle.

CONCLUSION

The complex and multifaceted process of post myocardial infarct behavior precludes the study of a single animal model as being fully representative of the changes occurring in patients. Information combined from both large and small animal myocardial infarct models might help unravel key pathophysiological, cellular and gene mechanisms and provide the foundation for testing potential therapeutic strategies. Careful consideration of an experimental protocol increases its translational value.

REFERENCES

(1) Klocke R, Tian W, Kuhlmann MT, Nikol S. "Surgical animal models of heart failure related to coronary heart disease." Cardiovasc Res. 2007 Apr 1;74(1):29-38.

(2) Rosenfeld ME, Averill MM, Bennett BJ, Schwartz SM. "Progression and disruption of advanced atherosclerotic plaques in murine models." CurrDrug Targets. 2008;9:210 –216.

(3) Systematic Eckle T, Grenz A, Köhler D, Redel A, Falk M, Rolauffs B, Osswald H, Kehl F, Eltzschig HK. "Evaluation of a novel model for cardiac ischemic preconditioning in mice." Am J Physiol Heart Circ Physiol. 2006 Nov;291(5):H2533-40.

(4) Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. "Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies." Physiol Genomics 16: 349–360, 2004.

(5) Salto-Tellez M, Yung Lim S, El-Oakley RM, Tang TP, Almsherqi ZA, Lim SK. "Myocardial infarction in the C57BL/6J mouse: a quantifiable and highly reproducible experimental model." Cardiovasc Pathol. 2004 Mar-Apr;13(2):91-7.

(6) Kumar D, Hacker TA, Buck J, Whitesell LF, Kaji EH, Douglas PS, Kamp TJ. "Distinct mouse coronary anatomy and myocardial infarction consequent to ligation." Coron Artery Dis. 2005 Feb; 16(1):41-4.

(7) Ahn D, Cheng L, Moon C, Spurgeon H, Lakatta EG, Talan MI. "Induction of myocardial infarcts of a predictable size and location by branch pattern probability-assisted coronary ligation in C57BL/6 mice." Am J Physiol Heart Circ Physiol. 2004 Mar; 286(3):H1201-7.

(8) Selye H, Bajusz E, Grassos S, Mendell P. "Simple techniques for the surgery occlusion of coronary vessels in the rat." Angiology. 1960; 11: 398-407.

(9) Spadaro J, Fishbein MC, Hare C, Pfeffer MA, Maroko PR. "Characterization of myocardial infarcts in the rat." Arch Pathol Lab Med. 1980; 104: 179-83.

(10) Taylor CB, Davis CB Jr, Vawter GF, Hass GM. "Controlled myocardial injury produced by a hypothermal method." Circulation 3: 239–253, 1951.

(11) Ciulla MM, Paliotti R, Ferrero S, Braidotti P, Esposito A, Gianelli U, Busca G, Cioffi U, Bulfamante G, Magrini F. "Left ventricular remodeling after experimental myocardial cryoinjury in rats." J Surg Res. 2004 Jan;116(1):91-7.

(12) van den Bos EJ, Mees BM, de Waard MC, de Crom R, Duncker DJ. "A novel model of cryoinjury-induced myocardial infarction in the mouse: a comparison with coronary artery ligation." Am J Physiol Heart Circ Physiol. 2005 Sep;289(3):H1291-300.

(13) Banerjee I, Fuseler JW, Price RL, Borg TK, Baudino TA. "Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse." Am J Physiol Heart Circ Physiol. 2007;293: H1883–H1891.

(14) Liu YH, Yang XP, Nass O, Sabbah HN, Peterson E, Carretero OA. "Chronic heart failure induced by coronary artery ligation in Lewis inbred rats." Am J Physiol 1997;272:H722–7.

(15) Kuhlmann MT, Kirchhof P, Klocke R, Hasib L, Stypmann J, Fabritz L, Stelljes M, Tian W, Zwiener M, Mueller M, Kienast J, Breithardt G, Nikol S. "G-CSF/SCF reduces inducible arrhythmias in the infracted heart potentially via increased connexin43 expression and arteriogenesis." J Exp Med. 2006 Jan 23;203(1):87-97.

(16) van den Borne SW, van de Schans VA, Strzelecka AE, Vervoort-Peters HT, Lijnen PM, Cleutjens JP, Smits JF, Daemen MJ, Janssen BJ, Blankesteijn WM. "Mouse strain determines the outcome of wound healing after myocardial infarction." Cardiovasc Res. 2009 Nov 1;84(2):273-82.

(17) Wehrens XH, Kirchhoff S, Doevendans PA. "Mouse electrocardiography: an interval of thirty years." Cardiovasc Res. 2000 Jan 1;45(1):231-7.

(18) Opitz CF, Mitchell GF, Pfeffer MA, Pfeffer JM. "Arrhythmias and death after coronary artery occlusion in the rat. Continuous telemetric ECG monitoring in conscious, untethered rats." Circulation. 1995 Jul 15;92(2):253-61.

(19) Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. "Myocardial infarct size and ventricular function in rats" Circ Res. 1979 Apr; 44(4):503-12.

(20) Zimmer HG, Gerdes AM, Lortet S, Mall G. "Changes in heart function and cardiac cell size in rats with chronic myocardial infarction." J Mol Cell Cardiol. 1990;22:1231–1243.

Hemodynamics of Cardiac Tissue Engineering

The heart has unique biomechanical properties. Each ventricular chamber (LV and RV) has a slightly different muscular/extracellular matrix (ECM) composition. The cellular composition of the heart also varies between species based on the amount of cardiomyocytes vs. supporting cells and on the amount and composition of the ECM (13). Studies show that cardiomyocytes behave poorly on man-made ECM surfaces (14). For that reason it is important to mimic the native cardiac mechanical environment.

The function of the supporting ECM is to stop or slow down the remodeling and scar formation process by preventing dilation of the heart muscle chambers. Meanwhile, delivered cells replace the dead cardiomyocytes/supporting cells and integrate with the neighboring cardiac tissue. The supporting ECM should have niches to sustain the survival of the delivered cells to facilitate regeneration process.

The definitive objective of post-MI therapy is to attenuate the remodeling process and regenerate the new cardiomyocyte/support cell-based muscle. This can be achieved by a cell delivery system consisting of a supporting matrix and suitable cells. Currently, most strategies fail to address all the important factors for successful regeneration including: the loss of cardiomyocytes, attacks of inflammatory cells on unprotected vulnerable tissue, cell isolation and expansion, immunogenicity of grafted cells or matrix, cell survival, biomechanical/electrical coupling properties of the tissue constructs, cytotoxicity levels and degradation properties.

The active mechanical function of cardiac tissue is mostly delivered during systole. If the myocardium is replaced by a noncompliant scar tissue, systolic contraction is decreased. At the same time, not only contraction but also relaxation (diastole) is affected by the inability to accommodate all of the blood volume inside the cavity as the heart chamber stiffens. Using invasive PV hemodynamic assessment within stages of regenerative therapy, especially the load-independent parameters and contractility, increases the ability to properly measure and compare pathophysiological cardiac function.

As delivered cells have very low retaining capacity, researchers are making a variety of biomaterial scaffolds. Biomaterials often exhibit an intrinsic stiffness that may compromise diastolic function. Biodegradation of the scaffold material(s) often remains incomplete, adding to the potential problems with diastolic function.

Diastolic dysfunction (DD) is characterized by myocardium that has decreased ability to generate force and is unable to accept an adequate volume of blood during diastole at normal diastolic pressure. This results in an inability to maintain stroke volume (SV). Degradation of scaffold causes:

- Poor relaxation (impaired lusitropy)
- Decreased compliance

DD occurs when these scaffold degradation processes are prolonged, slowed and/or incomplete. DD generally depends on the onset, rate and extent of decline of pressure in ventricles and the relationship between pressure and volume, stress, or strain during diastole.

PV CHARACTERISTICS OF DD (LV LOAD-DEPENDENT MEASUREMENTS)

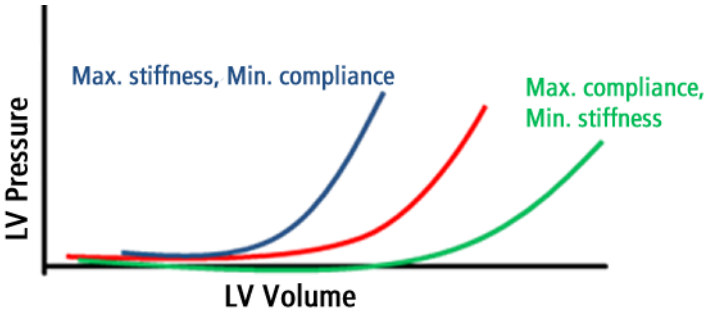
Representative PV loops of DD and diastolic failure can be found in “Understanding Lusitropy” on page 20

- LV EDP (end diastolic pressure) is increased with diastolic dysfunction as compared to healthy control. As the LV EDP rises left atrial and pulmonary venous pressures rise leading to pulmonary congestion and edema.
- Depending on the relative changes in SV and EDV, small decrease in EF and CO can be also observed. Because SV is decreased, decrease in ventricular SW can be also noticed on PV loop examination.
- Minimal/maximal rate of LV pressure change (dP/dt_{min}, dP/dt_{max}) is decreased.

Hemodynamics of Cardiac Tissue Engineering Cont.

PV CHARACTERISTICS OF DD (LV LOAD-INDEPENDENT MEASUREMENTS)

- EDPVR (end diastolic pressure volume relationship) represents relation between EDP-EDV points described by LV PV relationship. EDPVR is characterized by initial large increases in volume at low pressures. As volume increases further past the initial stage, pressure raises rapidly while volume increases slow as it is restrained by native ECM (e.g. collagen, proteoglycans, and glycoproteins). EDPVR curve fits are discussed in more depth in the publication by Burkhoff from 2005 (15). The EDPVR fits a non-linear curve that represents the diastolic stiffness (inverse of diastolic compliance) having the exponential fit EDP=A*exp (k*EDV), where k represents chamber stiffness or diastolic stiffness constant. K represents EDPVR slope, the change of ventricular pressure relative to a change in volume of the ventricular chamber (dP/dV).



Representative drawings of EDPVR curves. Diastolic dysfunction (DD) impacts load independent properties of the left ventricle (EDPVR) during cardiac diastole characterized by the compliance and stiffness.

- When a tissue engineered construct is attached to the LV, compliance of the chamber often decreases. This overall chamber stiffening leads to a decrease of myocardial relaxation properties called lusitropy. Lusitropy is characterized by unwinding of individual sheets of myocardium proceeding into partial or complete relaxation. For more about lusitropy please see “Understanding Lusitropy” on page 20. The unique lusitropic properties of myocardium change during heart development and aging further challenging tissue construct site selection and final implantation.
- Isovolumic relaxation time (IVR) is the time from aortic valve closure to mitral valve opening. During DD, IVR might be prolonged. IVR or Tau (isovolumic pressure decay) is caused by uncoupling helically woven layers of myocardial fibers (including extracellular matrix) assembled in linked sheets. Myocardial fiber arrangement generates unique (heart specific) relaxation patterns which account for observed IVR pressure gradient during LV emptying and filling. Therefore, IVR (Tau) will increase post-cardiac patch implantation as compared to a non-injured heart. For more information about IVR please see “Understanding Lusitropy” on page 20.
- Ventricle elastance (Ees) describes the transmission of mechanical energy from the ventricle into the arterial system. Effective arterial elastance (Ea) can be derived from the ratio of ESP to SV (Ea = ESP/SV). A healthy arterial system works with maximum coupling efficiency, where Ees/ Ea = 0.3 to 1.3 (16). However, values outside of this range have to be thoroughly examined before deemed pathological. This unit-less ratio of coupling (Ees/ Ea) increases during diastolic insufficiency since both the systolic and diastolic ventricular efficiencies decline while there is an increase in afterload.

Hemodynamics of Cardiac Tissue Engineering Cont.

Difficulties and Considerations for Development of Functional Myocardium

CELL SOURCE & TYPE

CATEGORY	TYPE	LIMITATION
Source of cells	Autologous	Difficult to harvest in numbers
	Allogenic	Immunology roadblocks
	Xenogeneic	Rejections
	Syngeneic	Cloning, limited translational value
Type of cells	Harvested primary cells	Difficult to expand/organ specific
	Secondary from cryopreserved cell banks	Immunology roadblocks
	Adult stem cells	Source and type to use
	Embryonic stem cells	Purification, potential malignancy

AMOUNT & METHOD OF CELLS TO DELIVER

Some investigators use delivery of isolated stem cells (1-6); others use in vitro-designed tissue equivalents (7-10). Cells delivered without a scaffold (intravascular, intracoronary, intramyocardial, transendocardial, epicardial) are prone to large losses. Within minutes, 85-90% of cells injected intravascularly are lost, almost all cells are trapped in lungs (11) with less than 1% found in heart (12). While larger animal model cell-retention rates are usually higher, an optimal delivery method is still elusive.

Isolated stem cell (derived-cardiomyocytes, skeletal myoblasts, fibroblasts, mesenchymal, adipose stem cells etc.) delivered directly to the infarct site have low cell survival and poor cell engraftment, due to a lack of functional vasculature at the implant site, inflammation, and constant tissue remodeling (7, 17).

TIMING OF CELL DELIVERY

Timing of cell delivery is impacted by animal model/physiology and therapeutic target (limiting scar extension, limiting inflammation, improving angiogenesis, vascularization). The best timing of implanted cells delivery is still under discussion as all above-mentioned factors that are in play (1, 5, 6, 8).

METHOD OF GROWING CELLS

Despite being able to grow functional cardiomyocytes in culture, the re-establishment of a contracting cardiac tissue (patch), including cardiac fibroblast and endothelial cells, is still elusive. Cardiac myocytes cultured in the standard 2D culture with the presence of growth-promoting medium lean towards de-differentiation and are often overgrown by non-myocytes. This has been largely overcome by using 3D culture environment. Additionally, the important influence of active or passive forces on cardiac myocyte growth, morphology, orientation, gene expression etc. has been demonstrated (7, 17). Substrates with a stiffness very close to that of the native adult rat myocardium were found to be favorable for heart cell morphology and function seen by cellular elongation, high contractile force and striations development (14).

Hemodynamics of Cardiac Tissue Engineering Cont.

TYPE OF SCAFFOLD

Most cardiac tissue engineering groups use scaffold proteins (e.g. collagen, gelatin, laminin, matrigel, hyaluronic acid (hyaluronan), alginate, and chitosan) or synthetic polymers (e.g. polylactic acid and polyglycolic acid) for tissue reconstitution from isolated cells.

Even the more common scaffolds, such as fibrin gel and matrigel, are far from ideal for cardiac tissue engineering. As the gelation rate of fibrin is slow and it lacks sufficient mechanical strength, there is a loss of delivered cells and low cell retention during injection. Its breakdown during heart contraction and relaxation is another downside along its high innate fibrinolysis rate. In case of matrigel, a biosafety concern exists, as it is derived from tumors (17).

Stabilization of the infarct area is the key concept for scaffold cellular delivery along with cell retention in the area. Temperature sensitive hydrogels mixed with variety of pro-angiogenic factors are a promising scaffold option (8).

SEEDING & GROWING CELLS ON SCAFFOLD

Tissue engineered cardiac patches (implanted tissue graft seeded with cardiomyocytes) have much less compact myocyte bundles as compared to native myocardium with less ability to generate necessary contractile force, often with non matured M bands. Core ischemia of the implanted graft seeded with cells often occurs. Cardiomyocytes incorporated on or in gelatin meshes form a thick cell layer only on the outside without a homogeneous cell distribution. Theoretical nutrient diffusion limit is 100-200 µm, but the limit is lower for more specialized cells such as cardiomyocytes (7). Substrate selection is also an important determinant of cell phenotypic development (14).

ELECTRICAL COUPLING

Despite observing implanted cell endurance and differentiation, mechanical and electrical cell-cell contacts between graft-and-host, required for synchronous contractions, are only rarely observed. Scar tissue appears to account for this problem by inhibiting contact between grafted cells and host tissue.

Inability to reproduce propagation of action potential (AP) is another concern. Action potential (current) propagates from SA (sinoatrial) pace-making node by intracellular channels (gap junctions). Most cells that are injected into the damaged SA node are not retained and might become pro-arrhytmic (18).

Hemodynamics of Cardiac Tissue Engineering Cont.

REFERENCES

(1) Li RK, Mickle DA, Weisel RD, Zhang J, Mohabeer MK. "In vivo survival and function of transplanted rat cardiomyocytes." Circ Res. 1996; 78: 283–288.

(2) Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE. "Regenerating functional myocardium: improved performance after skeletal myoblast transplantation." Nat Med. 1998; 4: 929–933.

(3) Xaymardan M, Sun Z, Hatta K, Tsukashita M, Konecny F, Weisel RD, Li RK. 'Uterine cells are recruited to the infarcted heart and improve cardiac outcomes in female rats." J Mol Cell Cardiol. 2012 Jun;52(6):1265-73.

(4) Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. "Bone marrow cells regenerate infarcted myocardium." Nature. 2001; 410: 701–705.

(5) Koh GY, Soonpaa MH, Klug MG, Pride HP, Cooper BJ, Zipes DP, Field LJ. "Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs." J Clin Invest. 1995; 96: 2034–2042.

(6) Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP. "Myoblast transplantation for heart failure." Lancet. 2001; 357: 279–280.

(7) Zimmermann WH, Schneiderbanger K, Schubert P, Didié M, Münzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. "Tissue engineering of a differentiated cardiac muscle construct." Circ Res. 2002 Feb 8;90(2):223-30.

(8) Wu J, Zeng F, Huang XP, Chung JC, Konecny F, Weisel RD, Li RK. "Infarct stabilization and cardiac repair with a VEGF-conjugated, injectable hydrogel." Biomaterials. 2011 Jan;32(2):579-86.

(9) Didié M, Christalla P, Rubart M, Muppala V, Döker S, Unsöld B, El-Armouche A, RauT, Eschenhagen T, Schwoerer AP, Ehmke H, Schumacher U, Fuchs S, Lange C, BeckerA, Tao W, Scherschel JA, Soonpaa MH, Yang T, Lin Q, Zenke M, Han DW, Schöler HR, Rudolph C, Steinemann D, Schlegelberger B, Kattman S, Witty A, Keller G, Field LJ, Zimmermann WH. "Parthenogenetic stem cells for tissue-engineered heart repair." J Clin Invest. 2013 Mar 1;123(3):1285-98.

(10) Fujita J, Itabashi Y, Seki T, Tohyama S, Tamura Y, Sano M, Fukuda K. "Myocardial cell sheet therapy and cardiac function." Am J Physiol Heart Circ Physiol. 2012 Nov 15;303(10):H1169-82.

(11) Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen HH, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BM, Wahl RL, Bulte JW. "Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction." Circulation. 2005 Sep 6;112(10):1451-61.

(12) Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. "Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution." Circulation. 2003 Aug 19;108(7):863-8.

(13) Banerjee I, Fuseler JW, Price RL, Borg TK, Baudino TA. "Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse." Am J Physiol Heart Circ Physiol. 2007;293: H1883–H1891

(14) Bhana B, Iyer RK, Chen WL, Zhao R, Sider KL, Likhitpanichkul M, Simmons CA, Radisic M. "Influence of substrate stiffness on the phenotype of heart cells." Biotechnol Bioeng. 2010 Apr 15;105(6):1148-60.

(15) Burkhoff D, Mirsky I, Suga H. "Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers." Am J Physiol Heart Circ Physiol. 2005 Aug;289(2):H501-12.

(16) De Tombe PP, Jones S, Burkhoff D, Hunter WC, Kass DA. "Ventricular stroke work and efficiency both remain nearly optimal despite altered vascular loading." Am J Physiol. 1993 Jun;264(6 Pt 2):H1817-24.

(17) Li Z and Guan J. "Hydrogels for Cardiac Tissue Engineering." Polymers 2011, 3(2), 740-761

(18) Macia E, Boyden PA. "Stem cell therapy is proarrhythmic." Circulation. 2009 Apr 7;119(13):1814-23.

Hemodynamic Assessment in Safety Pharmacology

Safety Pharmacology continues to be a rapidly developing discipline in a regulatory-driven process to generate data to inform risk/benefit assessment. In the cardiac field of study the aim of Safety Pharmacology (SP) is to characterize the pharmacodynamic/pharmacokinetic (PK/PD) relationship of a drug's adverse effects in the heart and circulation. Unlike toxicology, safety pharmacology includes a regulatory requirement to predict the risk of rare lethal events such as torsades de pointes (TdP) syndrome.

IMPORTANCE OF CARDIAC HEMODYNAMIC ASSESSMENT IN DRUG DISCOVERY AND EVALUATION

As an example of the importance of testing for potential adverse cardiovascular effects, in the mid 1990's the antihistamine, terfenadine (Seldane), was withdrawn following a growing awareness that the drug could evoke the potentially life threatening cardiac syndrome, torsades de pointes (TdP) or polymorphic ventricular tachycardia, in otherwise healthy patients (1). TdP reaction from this antihistamine only became evident after several millions of prescriptions, put many people at risk. This incident, and others like it in the late 1980s and early 1990s, gave rise to specialization of safety pharmacology, which was not previously recognized as very relevant. From this came safety testing for cardiovascular impact of non-cardiovascular drugs consisting of the evaluation of QT interval prolongation to screen for TdP liability.

Recent SP approaches using ECG measurements include measurements of QT interval as a surrogate biomarker of cardiac biopotentials (1, 2, 9). For example, non-cardiovascular indicated drugs such as droperidol, lidoflazine, and cisapride were removed from market solely based on the QT interval. Clinicians are constantly faced with both older and newly approved drugs labelled as potentials for TdP. While at the same time drugs considered to be safe in non-clinical studies may be found to have QT-liability (proarrhythmic risk) in early clinical studies (2). Regulatory authorities might ask for scientific rationalization for this incongruity and, as long as the safety of subjects in later phase clinical trials is assured, the sponsor may then decide whether to continue or terminate the drug development in light of the risks.

SAFETY PHARMACOLOGY GUIDELINES

The S7A: Safety Pharmacology Studies for Human Pharmaceuticals guideline for the conduct of safety pharmacology evaluations recommends using a core battery of studies on three vital organ systems (cardiovascular, respiratory and central nervous system) to assess the potential risks of novel pharmaceuticals for human use.

Core Cardiovascular Studies:

- Central arterial pressure
- Heart rate
- Electrocardiogram (ECG)
- Electrophysiology (hERG)

Supplemental Cardiovascular Studies:

- Cardiac output
- Ventricular contractility
- Ventricular resistance

TRADITIONAL METHODS OF ASSESSMENT IN PRE-CLINICAL SAFETY PHARMACOLOGY

Cardiac and hemodynamic studies have the potential for significant variation between investigators as the ability to detect adverse effects is subjective and sometimes inconsistent. For detecting adverse effects of tested (candidate) drug on cardiac contractility, pre-clinical safety pharmacology mostly uses a combination of ECG with a pressure catheter to study QA interval compared with LV dP/dt max (Fig. 1). This set up allows for a two-index comparison of heart contractility to assess the inotropic induction (3, 4, 5). The QA interval covers the period of time of initial depolarization of ventricles (R decline) until the time when the aortic valve opens. For the purpose of determining the QA interval, the time between the Q on the ECG and the beginning of the upstroke on the arterial blood pressure is used as an indicator of altered LV contractility (Fig. 1). Drugs that slow conduction velocity through the heart could prolong the QA, and may have more effect on QA than on LV dP/dt max. Theoretically, reduction of blood

REFERENCES

(1) Monahan BP, Ferguson CL, Killeavy ES, Lloyd BK, Troy J, Cantilena LR., Jr "Torsades de pointes occurring in association with terfenadine use." JAMA. 1990;264:2788–2790.

(2) Shah RR. "If a drug deemed 'safe' in nonclinical tests subsequently prolongs QT in phase 1 studies, how can its sponsor convince regulators to allow development to proceed?" Pharmacol Ther. 2008 Aug;119(2):215-21.

Hemodynamic Assessment in Safety Pharmacology Cont.

pressure induced by the candidate drug causing reduced arterial stiffness might reduce pulse wave propagation and thus QA interval. Moreover, QA captures reduced contractility more precisely than compared to increased contractility.

Other safety pharmacologists use the LV dP/dt max-HR relationship to determine the force-frequency relationship while singling out LV dP/dt max as a measure of pharmacodynamic heart contractility endpoint (6). It is important to note that many drugs induce both chronotropic and inotropic effects which changes HR along with LV dP/dt max and that LV dP/dt max changes with HR. Thus changes to contractility must be evaluated based on the corresponding changes in heart rate (6).

Dp/dt measurements are sensitive to changes in LV pre-load (10). In mice for example, it has been shown that a change in LV preload as low as 1 mmHg can have an 18% change in the dP/dt readings (11). Detecting the onset of a small change in preload requires extremely stable pressure sensors with a resolution greater than 1 mmHg.

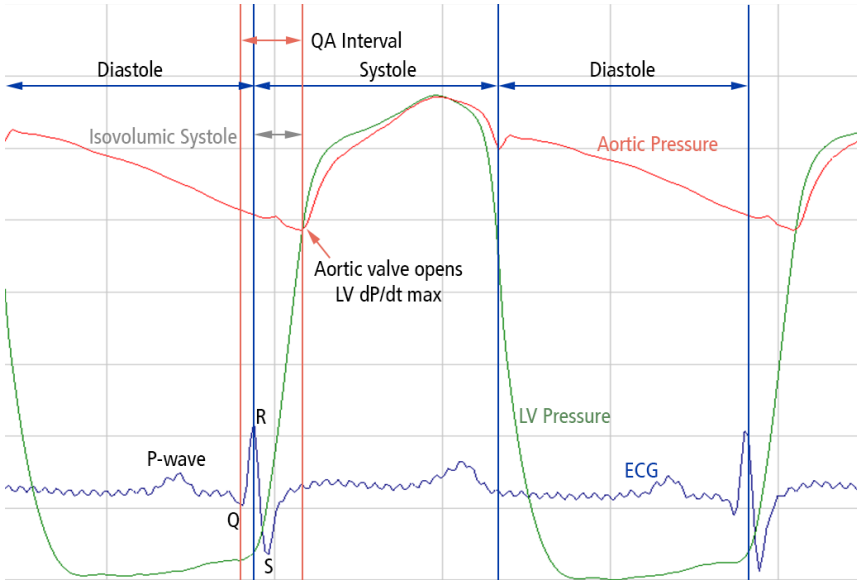


Fig. 1: QA interval approximates the average rate of isovolumic systolic pressure development.

PV LOOPS IN PRE-CLINICAL SAFETY PHARMACOLOGY

Recently, Conductance and Admittance PV loop technology has been used for screening and pharmacologic assessment of the inotropic state of the heart (7, 8). While this method is the gold standard for investigation of cardiac function with respect to clinical disease, it is currently not widely used in safety pharmacology (7). By using the relationship of pressure and volume for interrogation of the LV, safety pharmacologists can better determine lusitropic effects of myocardial relaxation that cannot be otherwise captured by using two indices comparison (e.g. QA interval with LV dP/dt max). Moreover, other important parameters of load independent measurements such as ESPVR, EDPVR, PRSW, PVA can be determined, strengthening conclusions about the tested drug candidate.

REFERENCES CONT.

(3) Mooney L, Marks L, Philp KL, Skinner M, Coker SJ, Currie S. "Optimising conditions for studying the acute effects of drugs on indices of cardiac contractility and on haemodynamics in anaesthetized guinea pigs." J Pharmacol Toxicol Methods. 2012 Jul;66(1):43-51.

(4) Johnson DM, Geys R, Lissens J, Guns PJ. "Drug-induced effects on cardiovascular function in pentobarbital anesthetized guinea-pigs: invasive LVP measurements versus the QA interval." J Pharmacol Toxicol Methods. 2012 Sep;66(2):152-9.

(5) Norton K, Iacono G, Vezina M. "Assessment of the pharmacological effects of inotropic drugs on left ventricular pressure and contractility: an evaluation of the QA interval as an indirect indicator of cardiac inotropism." J Pharmacol Toxicol Methods. 2009 Sep-Oct;60(2):193-7.

(6) Markert M, Trautmann T, Groß M, Ege A, Mayer K, Guth B. "Evaluation of a method to correct the contractility index LVdP/dt(max) for changes in heart rate." J Pharmacol Toxicol Methods. 2012 Sep;66(2):98-105.

(7) Marks L, Borland S, Philp K, Ewart L, Lainée P, Skinner M, Kirk S, Valentin JP. "The role of the anaesthetised guinea-pig in the preclinical cardiac safety evaluation of drug candidate compounds." Toxicol Appl Pharmacol. 2012 Sep 1;263(2):171-83.

(8) Violin JD, DeWire SM, Yamashita D, Rominger DH, Nguyen L, Schiller K, Whalen EJ, Gowen M, Lark MW. "Selectively engaging β-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance." J Pharmacol Exp Ther. 2010 Dec;335(3):572-9.

(9) Authier S, Gervais J, Fournier S, Gauvin D, Maghezzi S, Troncy E. "Cardiovascular and respiratory safety pharmacology in Göttingen minipigs: Pharmacological characterization." J Pharmacol Toxicol Methods. 2011 Jul-Aug;64(1):53-9.

(10) Kass DA, Maughan WL, Guo ZM, Kono A, Sunagawa K, Sagawa K. Comparative influence of load versus inotropic states on indexes of ventricular contractility: experimental and theoretical analysis based on pressure volume relationships. Circulation. 1987;76:1422–1436.

(11) Georgakopoulos D, Mitzner WA, Chen CH, Byrne BJ, Millar HD, Hare JM, Kass DA. In vivo murine left ventricular pressure-volume relations by miniaturized conductance-micromanometry. Am J Physiol. 1998 Apr;274(4 Pt 2):H1416-22.

Right Ventricle Pressure-Volume Loops

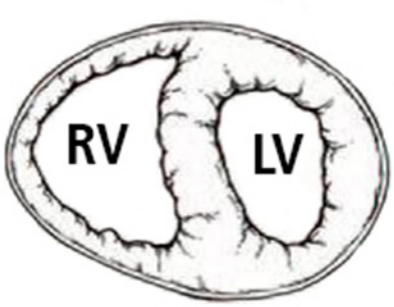
As hemodynamic assessment techniques progress, RV pathology can be better characterized including assessing changes from a healthy to a compensated (hypertrophied) condition and then decompensated condition. Cardiac catheterization remains the best method to diagnose pulmonary hypertension, assess disease severity, and determine prognosis and response to therapies. By directly measuring pressures and volumes, right heart catheterization allows determination of prognostic systolic indices, diastolic indices, pulmonary vascular indices and the coupling ratio. For more information about using right ventricular PV loops to study pulmonary artery hypertension see: "Pulmonary Artery Hypertension & RV PV Loops" on page 86.

Primarily, RV function may be impaired due to:

- Primary right side heart disease
- Secondary LV issues
- Heart valve disease (RV or LV)
- Post-reparative changes from congenital heart disease (e.g. Fallot tetralogy, pulmonary stenosis, insufficiency)
- Post-correction of hypoplastic left heart syndrome
- Arterio-caval shunt (increased afterload)

RV dysfunction may affect LV function by:

- Limiting LV preload
- Systolic and diastolic interaction through intra-ventricular septum
- Pericardium ventricular interdependence by constraints of heart pericardium post-inflammation



The anatomical and physiological characteristics of a typical right ventricle is complex. Compared to the ellipsoidal LV shape, the RV appears triangular when viewed from the side and crescent shaped when viewed in cross section (above). The RV shape is also influenced by the position of the inter-ventricular septum. Under normal loading and electrical conditions, the septum is concave toward the LV in both systole and diastole. RV has different genetic composition as compared to the LV, making it vulnerable when hypertrophied due to its septomarginal muscular band, which can divide the ventricle into 2 chambers (double-chambered RV). Additionally, the volume of the RV is larger than the volume of the LV, whereas RV mass is approximately one sixth that of the LV. This allows better accommodation of volume but not pressure. A sudden increase in RV pressure is deadly, as compared to a slow afterload increase since RV adaptation occurs at a slower pace.

Figure from Champion HC, et. al. "Comprehensive Invasive and Noninvasive Approach to the Right Ventricle–Pulmonary Circulation Unit: state of the art and clinical and research implications." Circulation. 2009 Sep 15;120(11):992-1007

METHODS FOR ASSESSING RV HEMODYNAMICS

Non-Invasive Methods:

- Echocardiography (2D or 3D)
- Tissue Doppler imaging (TDI)
- Magnetic resonance imaging (MRI)
- Computed (large animals) or Micro-computed (rodents) Tomography; CT or micro-CT

Invasive Methods:

- Virtual pressure-volume loops (MRI and Pressure)
- Catheter measurement of pulmonary artery pressure (PAP), Right ventricular (RVP) and right atrial pressures (RAP).
- Pressure Catheter combined with Perivascular Flowprobe
- Right ventricle pressure volume (RV-PV) loops (Pressure-volume catheter)

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Right Ventricle Pressure-Volume Loops Cont.

METHODS FOR ASSESSING RV HEMODYNAMICS CONT.

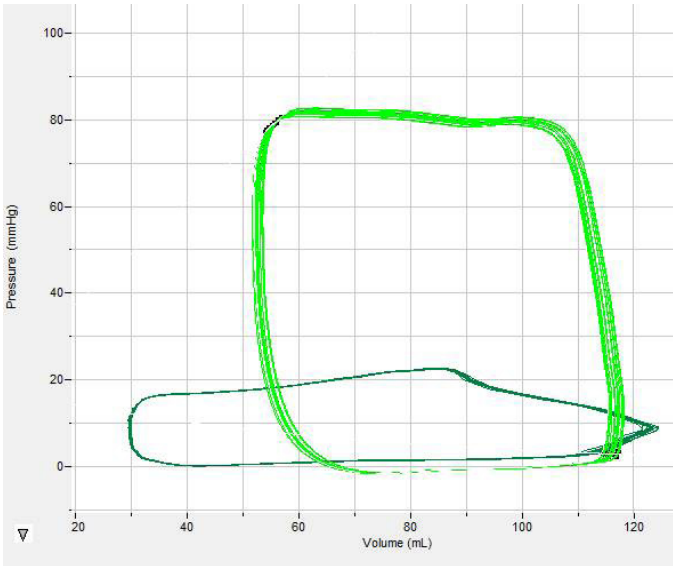
Each method has advantages and limitations so it is important to consider what parameters are of interest, study methodology requirements, equipment requirements and cost, measurement time, difficulty and repeatability. For a comparison between Echocardiography, PV Catheterization, CT and MRI see "Introduction: Cardiac Volume Measurement Methods" on page 2.

Note: Major difficulties of basic RV 2D echocardiography as compared to the basic LV 2D echo include:

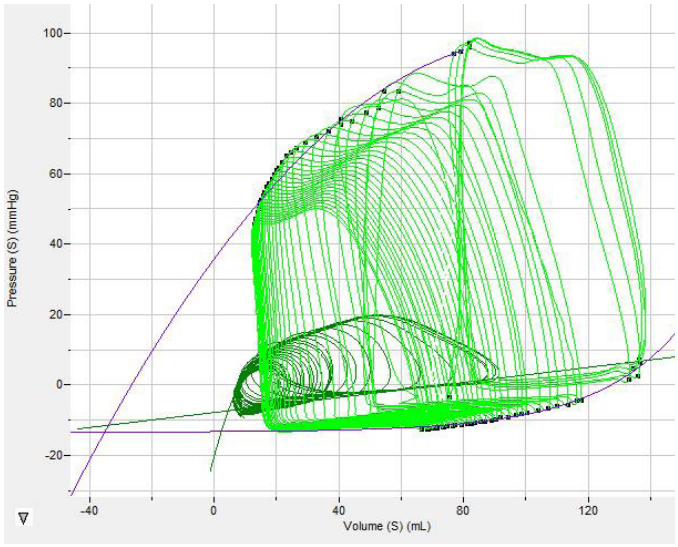
- Inadequate formula to characterize RV volume based on the RV shape. Current formulas specific to the LV.
- As the RV has thinner walls and chamber has more trabeculations as compared to the LV, it is more complex to echocardiographically characterize it.
- An acoustic barrier is created by its retrosternal position.

RIGHT VENTRICLE PRESSURE-VOLUME LOOP CHARACTERISTICS AS COMPARED TO THE LEFT VENTRICLE

- EDPVR of the RV is characterized by its high compliance (compliance is increased) as compared to the LV.
- At any given end-diastolic volume (EDV) the RV end-diastolic pressure (EDP) is lower, making the final EDPVR shallower as compared to the LV.
- Ejection of blood into the highly compliant, low-resistance pulmonary circulation results in better coupling (Ees/Ea).
- Normal RV generates less than 20% of the stroke work (SW) of the LV while moving the same volume of blood. Compared to the LV, a much lower proportion of RV SW goes to pressure generation.



Pressure-Volume loops during steady state recording. Images are taken from the same sheep heart. Dark green PV loops represent RV steady state, while light green are LV PV loops.



Pressure-Volume loops during IVC occlusion. Images are taken from the same pig heart. Dark green PV loops represent RV, while light green are LV PV loops post-IVC occlusion.

Right Ventricle Pressure-Volume Loops Cont.

RIGHT VENTRICLE PRESSURE-VOLUME LOOP CHARACTERISTICS AS COMPARED TO THE LEFT VENTRICLE CONT.

- The RV accommodates dramatic variations in venous return resulting from changes in volume status, position, and respiration while maintaining more or less constant cardiac output (CO). In part this is because the thin RV is easily distensible, but to a larger extent it is a direct result of RV geometry. Like the LV, the RV utilize the Frank-Starling mechanism to increase SW as a consequence of an increase in RV stretch, but much larger changes in RV volume are needed before the Frank-Starling mechanism is engaged.
- The RV coronary perfusion pattern significantly differs from that of the LV. Because tissue pressure in the LV rises during systole to systemic levels, coronary perfusion of the LV is largely confined to the diastolic interval. Tissue pressure in the RV does not normally exceed aortic root systolic pressure, permitting continued coronary flow throughout the cardiac cycle. Thus, under typical hemodynamic conditions, coronary flow to the RV is roughly balanced between systolic and diastolic time periods.

RV INDICES IN MICE (Tabima et. al. 2010)			
INDICES	**HEALTHY RV**	**HYPERTROPHIED RV**	**FORMULA**
Heart rate (HR)	611 ± 31 bpm	636 ± 31 bpm	
RV Systolic pressure	27 ± 3 mmHg	45 ± 17 mmHg	
RV Diastolic pressure	1.4 ± 0.9 mmHg	2.7 ± 1.4 mmHg	
RV Stroke work (SW)	386 ± 76 mmHg*µL	926 ± 265 mmHg*µL	SV*MAP
RV Cardiac output (CO)	8.5 ± 2.3 ml/min	5.2 ± 2.6 ml/min	SV*HR
RV Stroke volume (SV)	13.9 µL	8.2 µL	CO/HR (EDV-ESV)
Ejection Fraction (EF)	51 ± 11%	28 ± 13%	SV/EDV*100
Mean Arterial pressure (MAP)	27.8 mmHg	112.9 mmHg	SW/SV
dP/dt_{max}	2522 ± 660 mmHg/s	3164 ± 826 mmHg	
dP/dt_{max} - end-diastolic volume	84 ± 17 mmHg/s/µL	177 ± 93 mmHg/s/µL	dP/dt_{max} vs. EDV
dP/dt_{min}	-1971 ± 499 mmHg/s	-3009 ± 1120 mmHg/s	
Preload recruitable stoke work (PRSW)	20.9 ± 5.6 mmHg	33.9 ± 5.9 mmHg	SW vs. EDV
Ventricular end-systolic elastance (Ees)	1.8 ± 0.5 mmHg/µL	2.4 ± 0.2 mmHg/µL	ESP/ESV
Effective arterial elastance (Ea)	2.7 ± 1.2 mmHg/µL	16.4 ± 2.5 mmHg/µL	ESP/SV
Coupling ratio: Ees/Ea	0.71 ± 0.27 Optimal > 0.5	0.35 ± 0.17 Uncoupled < 0.5	SV/ESV

REFERENCES

Andersen A, et. al. "Effects of phosphodiesterase-5 inhibition by sildenafil in the pressure overloaded right heart." Eur J Heart Fail. 2008 Dec;10(12):1158-65.

Bartelds B, et. al. "Bartelds Differential responses of the right ventricle to abnormal loading conditions in mice: pressure vs. volume load." Eur J Heart Fail. 2011 Dec;13(12):1275-82.

Blaudszun G, Morel DR. "Superiority of desflurane over sevoflurane and isoflurane in the presence of pressure-overload right ventricle hypertrophy in rats." Anesthesiology. 2012 Nov; 117(5): 1051-61.

Boissiere J, et. al. "Doppler tissue imaging in assessment of pulmonary hypertension-induced right ventricle dysfunction." Am J Physiol Heart Circ Physiol. 2005 Dec;289(6):H2450-5.

Fitzpatrick JM, Grant BJ. "Effects of pulmonary vascular obstruction on right ventricular afterload." Am Rev Respir Dis. 1990 Apr;141:944-52.

Kumar S, et. al. "Cardiac-specific genetic inhibition of nuclear factor-κB prevents right ventricular hypertrophy induced by monocrotaline." Am J Physiol Heart Circ Physiol. 2012 Apr 15; 302(8): H1655-66.

Piao L, et. al. "GRK2-mediated inhibition of adrenergic and dopaminergic signaling in right ventricular hypertrophy: therapeutic implications in pulmonary hypertension." Circulation. 2012 Dec 11; 126(24): 2859-69.

Tabima DM, Hacker TA, Chesler NC. "Measuring right ventricular function in the normal and hypertensive mouse hearts using admittance-derived pressure-volume loops." Am J Physiol Heart Circ Physiol. 2010 Dec; 299(6): H2069-75.

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Pulmonary Artery Hypertension & RV PV Loops

PULMONARY HYPERTENSION AND RIGHT VENTRICLE REMODELING

The pulmonary circulation has shorter arteries and veins, more distensible (high compliant and low resistant) large arteries, and a larger number of peripheral arteries as compared to the systemic circulation. This leads to RV afterload being significantly lower than LV afterload and matching ventricular-vascular coupling. Pulmonary hypertension (PH) is diagnosed when mean pressure in the pulmonary artery increases over a threshold. Pulmonary artery hypertension (PAH) is a subtype of PH classification defined as a mean pulmonary artery pressure (PAP) greater than 25 mmHg with peak pressure greater than 33 mmHg at rest or mean PAP greater than 30 mmHg during exercise (1, 19). Other clinically distinct PH causes include: PH owing to the left heart disease, PH owing to lung disease or hypoxia, and chronic thromboembolic PH (CTEPH).

PAH is important subtype of PH as it originates when pulmonary arteries muscularize while its vascular media hypertrophies, leading to a progressive narrowing of the arterial lumen. It is a syndrome in which obstruction of pulmonary arteries increases pulmonary vascular resistance (PVR) leading to right ventricular (RV) hypertrophy (20). Its etiology is not completely understood at this time, as multiple factors are involved. Vascular pathology changes induced by PH are characterized by intimal thickening and fibrosis, medial hypertrophy, muscularization of previously non-muscularized arteries, adventitial proliferation and increased extracellular matrix (ECM) deposition (8). PAH is responsible for proximal pulmonary artery stiffening (changes of PA pulsatility, compliance, capacitance, distensibility, elastic modulus, and pressure-independent stiffness index beta) (2) and RV dysfunction (18). RV has to accommodate (the compensatory changes) an increased afterload due to the PA pressure elevation or it will fail.

Experimental models of pulmonary hypertension include direct damage of pulmonary endothelial cells (EC) by monocrotalin, alpha-napthyltiourea, microspheres, Angiopoetin-1, or Bleomycin, and subsequent EC and vascular smooth muscle cells proliferation in the area (19). In this context, RV autoregulation changes promote a temporary increase in RV contractility. During this condition, elevated wall stresses develop in the RV lateral free wall (4) and outflow tract (5). Increased wall stresses from increased mean pressure in the PA and increased pulmonary vascular resistance (PVR) further stimulate RV hypertrophy (5) and RV free wall fibrosis (6).

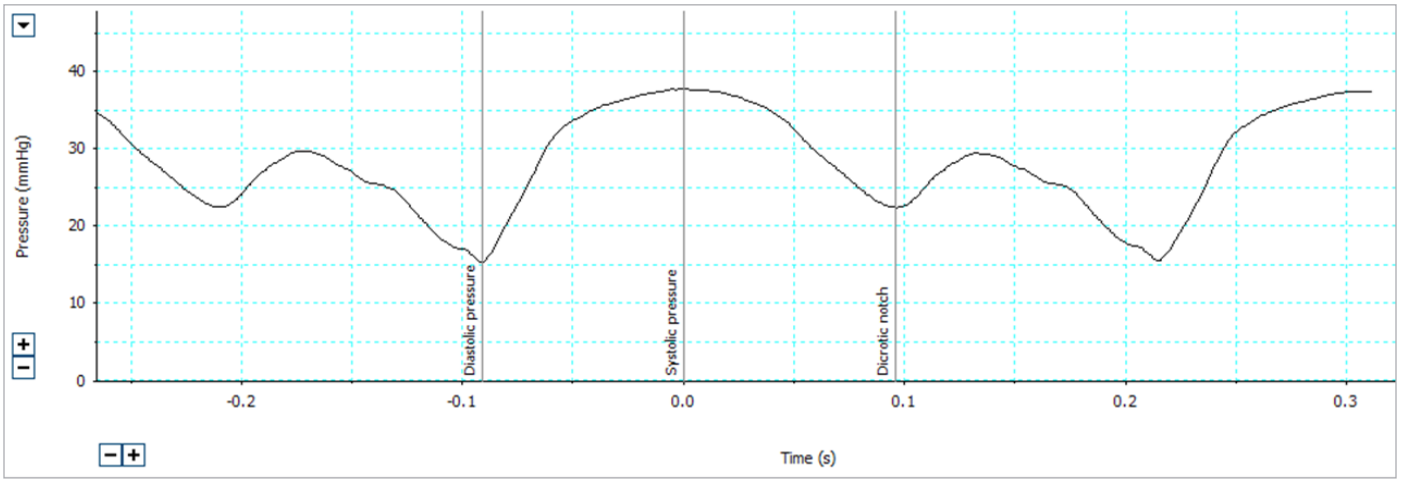


Fig. 1: Pressure trace in pig pulmonary artery during hypertension. Peak pressure is over 35 mmHg at rest.

Pulmonary Artery Hypertension & RV PV Loops Cont.

ASSESSMENT OF RIGHT VENTRICLE POST PAH USING RV PV LOOPS

Based on experimental data obtained from mouse RV pressure-volume study of PAH injury, models of the ventricular mechanics showed increasing RV afterload while effectively putting strain on the RV. The models show that vascular resistance, arterial elastance and arterial narrowing all play important roles in final RV remodeling (21).

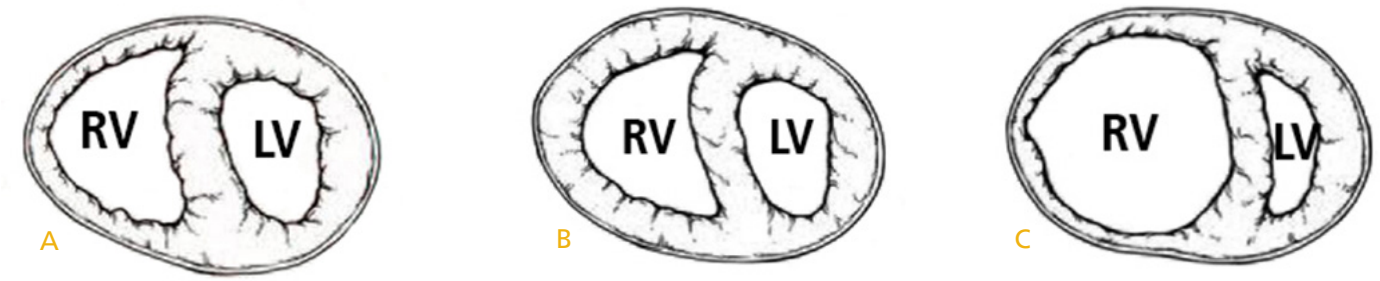


Fig. 2: A) Intact Rigth Ventricle B) PAH compensatory changes are responsible for RV free wall hyperthrophy which temporarily protects RV cardiac output and stroke volume. Heart rate might temporarily increase. RV afterload increases without significant volume changes (Fig. 3A). As PAH progresses changes in RV chamber volume occur (Fig. 3B) C) Right Ventricle dilates and fails, decrease in stroke work, stroke volume, cardiac output, and ejection fraction (Fig. 3C). Figures from Champion HC, et. al. Comprehensive Invasive and Noninvasive Approach to the Right Ventricle–Pulmonary Circulation Unit: state of the art and clinical and research implications. Circulation. 2009 Sep 15;120(11):992-1007

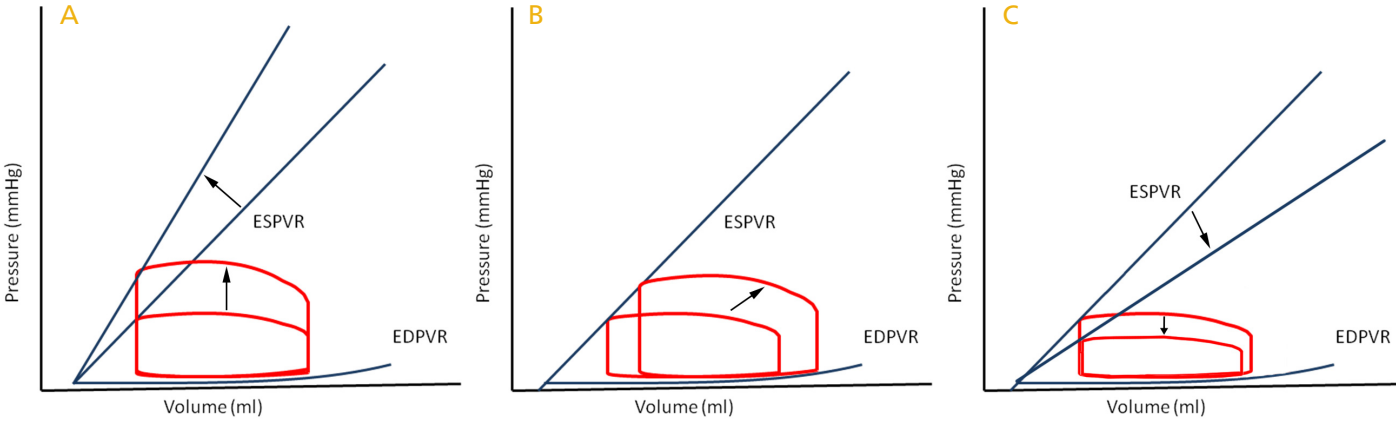


Fig. 3: Representative drawing of RV PV loops post PAH. A) Represents situation of PAH where temporary increase of pressure in the PA (increase of after-load) can be detected in the RV PV loop without volume changes. B) Represents compensatory changes of RV (increase of after-load) where both pressure and volumes changes during PAH. C) Represents failing RV as chamber dilates which is marked by decrease of stroke work, cardiac output and ejection fraction and other key parameters.

ASSESSMENT OF RIGHT VENTRICLE POST PAH USING RV PV LOOPS CONT.

In the mouse, the onset of PAH is characterized by significant RV ESP increase. During early PAH the right ventricle increases its efficiency during systole to meet an increasing afterload, but as PAH progresses, its work efficiency plateaus (Fig. 3A) (22). During diastole RV EDV increases, suggesting a larger RV compliance, but in fact, as RV EDP increases, it also makes the RV chamber stiffer in diastole and that is most likely a consequence of RV wall stiffening (22). Because increased RV EDV is a strong predictor of mortality in PAH (7) detection of changes using pressure-volume measurements are very important.

Since the mechanism(s) and factors involved in the transition from an adaptive hypertrophy to maladaptive remodelling are currently unknown, a comprehensive study of RV hemodynamics is valuable. More research is underway to assess RV hemodynamics, energy balance, LV-RV dysynchrony and other possible mechanisms leading to

Pulmonary Artery Hypertension & RV PV Loops Cont.

this progressive RV failure and multiple studies in recent years used RV PV loops in their pulmonary hypertension research (9-17). For an excellent in-depth review of *in-vivo* measurements and detection of stiffening of the PA influencing the RV hemodynamics in clinical, large animal and small animal setting, please refer to an article by Chesler & Tian (18).

Historically, RV mass measured by non-invasive magnetic resonance imaging has been used as an indication of RV dysfunction due to PAH. This method, however, is not considered a strong predictor of mortality, and for this reason, this finding cannot be fully translated into the clinic (7). This disconnect between mass and mortality is possibly due to an adaptive remodelling, known also as a concentric hypertrophy without dilation happening in the RV post PAH. This leads to more longitudinal monitoring of RV pressure and volume during PAH.

One of the first uses of 5F and 6F Conductance Catheters was described by Dickstein *et. al.* in 1995. This group measured volume in the RV and correlated data to flow derived volumes measured by 16 mm Transonic Flowprobe placed on the pulmonary artery (3). The advances of Admittance technology reduces the geometric dependence of PV measurements and has allowed for right ventricular pressure-volume measurements to be more precise (15, 16).

REFERENCES

(1) Simonneau G, et. al. "Updated clinical classification of pulmonary hypertension." J Am Coll Cardiol. 2009 Jun 30;54(1 Suppl):S43-54.

(2) Sanz J, et. al. "Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance." JACC Cardiovasc Imaging. 2009 Mar;2(3):286-95

(3) Dickenstein ML, et. al. "Assessment of right ventricular contractile state with the conductance catheter technique in the pig." Cariovasc. res 1995; 29:820-6

(4) Quaife RA, et. al. "Importance of right ventricular end-systolic regional wall stress in idiopathic pulmonary arterial hypertension: a new method for estimation of right ventricular wall stress." Eur J Med Res. 2006 May 5;11(5):214-20.

(5) Simon MA, et. al. "Phenotyping the right ventricle in patients with pulmonary hypertension." Clin Transl Sci. 2009 Aug;2(4):294-9.

(6) Sanz J, et. al. "Prevalence and correlates of septal delayed contrast enhancement in patients with pulmonary hypertension." Am J Cardiol. 2007 Aug 15;100(4):731-5.

(7) van Wolferen SA, et. al. "Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension." Eur Heart J. 2007 May; 28(10):1250-7.

(8) Stenmark KR, et. al. "The adventitia: essential role in pulmonary vascular remodeling." Compr Physiol. 2011 Jan;1(1):141-61.

(9) Wagenaar GT, et. al. "Ambrisentan reduces pulmonary arterial hypertension but does not stimulate alveolar and vascular development in neonatal rats with hyperoxic lung injury." Am J Physiol Lung Cell Mol Physiol. 2013 Feb 15;304(4):L264-75.

(10) Cavasin MA, et. al. "Selective class I histone deacetylase inhibition suppresses hypoxia-induced cardiopulmonary remodeling through an antiproliferative mechanism." Circ Res. 2012 Mar 2;110(5):739-48.

(11) Umar S, et. al. "Allogenic stem cell therapy improves right ventricular function by improving lung pathology in rats with pulmonary hypertension." Am J Physiol Heart Circ Physiol. 2009 Nov;297(5):H1606-16.

(12) Kumar S, et. al. "Cardiac-specific genetic inhibition of nuclear factor-κB prevents right ventricular hypertrophy induced by monocrotaline." Am J Physiol Heart Circ Physiol. 2012 Apr 15;302(8):H1655-66.

(13) Wang Z, et. al. "Effects of collagen deposition on passive and active mechanical properties of large pulmonary arteries in hypoxic pulmonary hypertension." Biomech Model Mechanobiol. 2013 Nov;12(6):1115-25.

(14) Nagendran J, et. al. "Endothelin axis is upregulated in human and rat right ventricular hypertrophy." Circ Res. 2013 Jan 18;112(2):347-54.

(15) Tabima DM, et. al. "Measuring right ventricular function in the normal and hypertensive mouse hearts using admittance-derived pressure-volume loops." Am J Physiol Heart Circ Physiol. 2010 Dec;299(6):H2069-75.

(16) Schreier D, et. al. "The role of collagen synthesis in ventricular and vascular adaptation to hypoxic pulmonary hypertension." J Biomech Eng. 2013 Feb;135(2):021018.

(17) Yasuda T, et. al. "Rho-kinase inhibition alleviates pulmonary hypertension in transgenic mice expressing a dominant-negative type II bone morphogenetic protein receptor gene." Am J Physiol Lung Cell Mol Physiol. 2011 Nov;301(5):L667-74.

(18) Tian L, Chesler NC. "In vivo and in vitro measurements of pulmonary arterial stiffness: A brief review. "Pulm Circ. 2012 Oct;2(4):505-17.

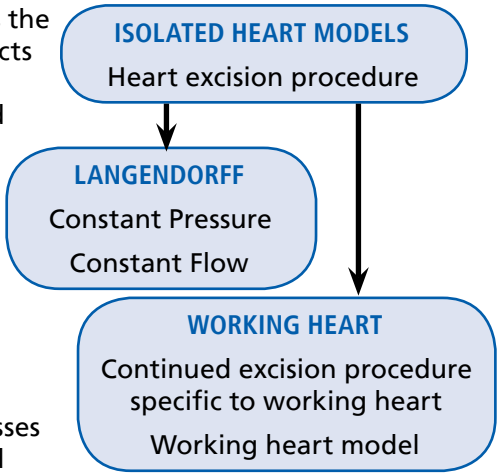
(19) Marsboom GR, Janssens SP. "Models for pulmonary hypertension. Drug Discovery Today Disease models." 2004 1(3): 289-96.

(20) Ciuclan L, et. al. "A novel murine model of severe pulmonary arterial hypertension." Am. J. Respir.Crit.CareMed. 2011: 184, 1171–1182.

(21) Tewari SG, et. al. "Analysis of cardiovascular dynamics in pulmonary hypertensive C57BL6/J mice." Front in Physiol 2013 Dec; (4), 1-9

(22) Wang Z,et. al. "Progressive right ventricular functional and structural changes in a mouse model of pulmonary arterial hypertension." Physiol Rep, 1 (7), 2013.

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

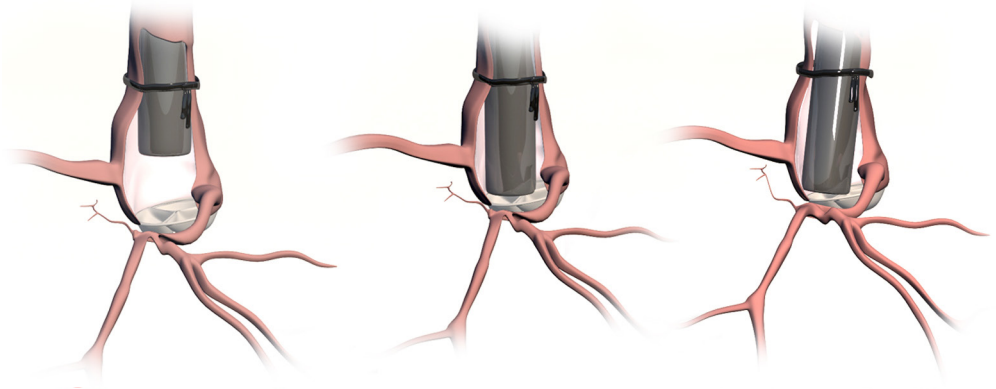
SUGGESTED GUIDELINES BY SPECIES

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

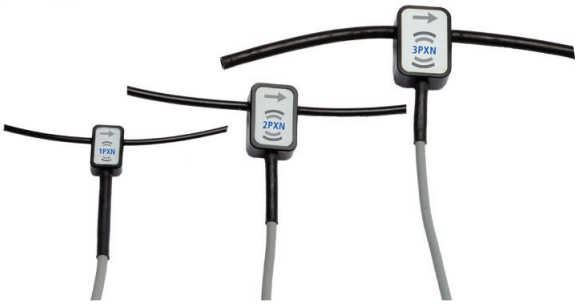
Aortic cannula with groove incorrectly placed in the aortic arch

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

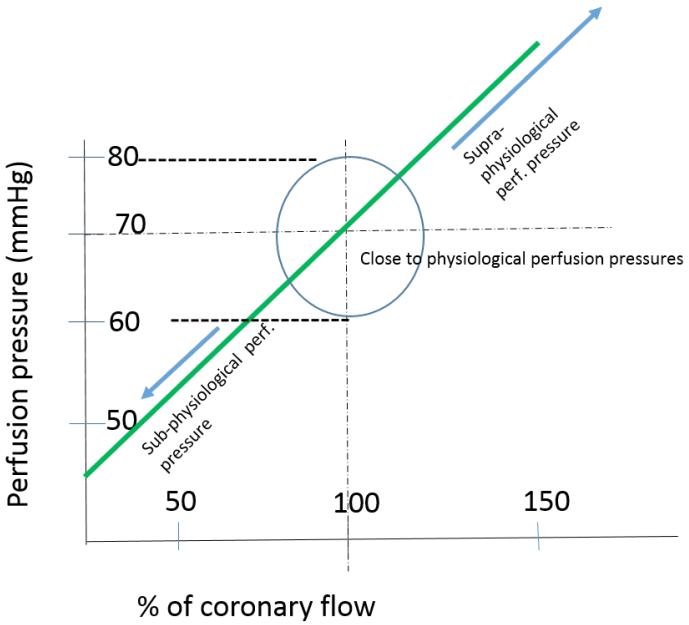
Langendorff Heart Model for Cardiac Assessment Cont.

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

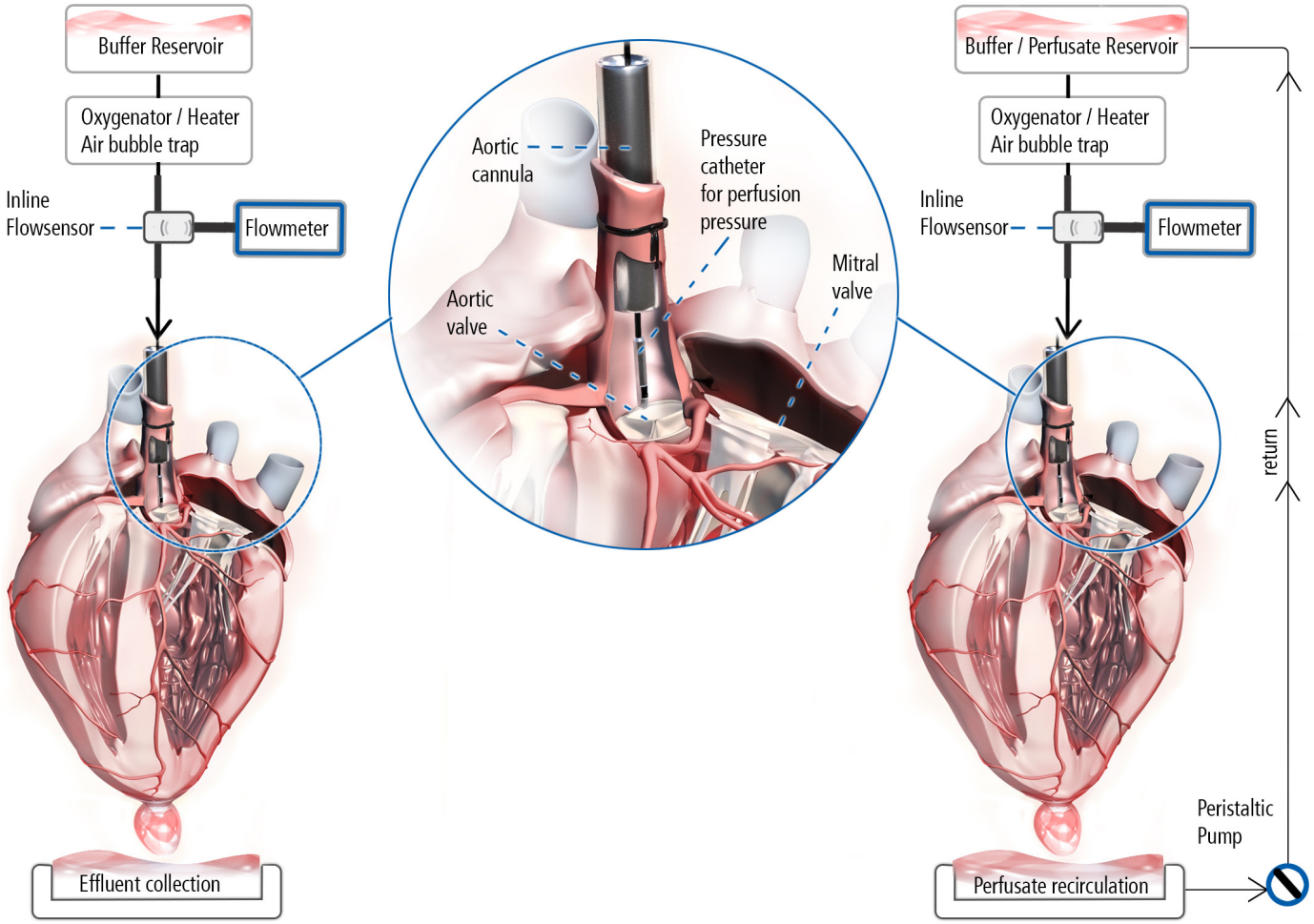
Flow is held constant at fixed (known) flow rate via a peristaltic pump and changes in coronary resistance are detected as changes in pressure. This set up is predominantly used when studying coronary vascular tone (vasoconstrictors, vasodilators), and smooth muscle or endothelial function. The peristaltic 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.



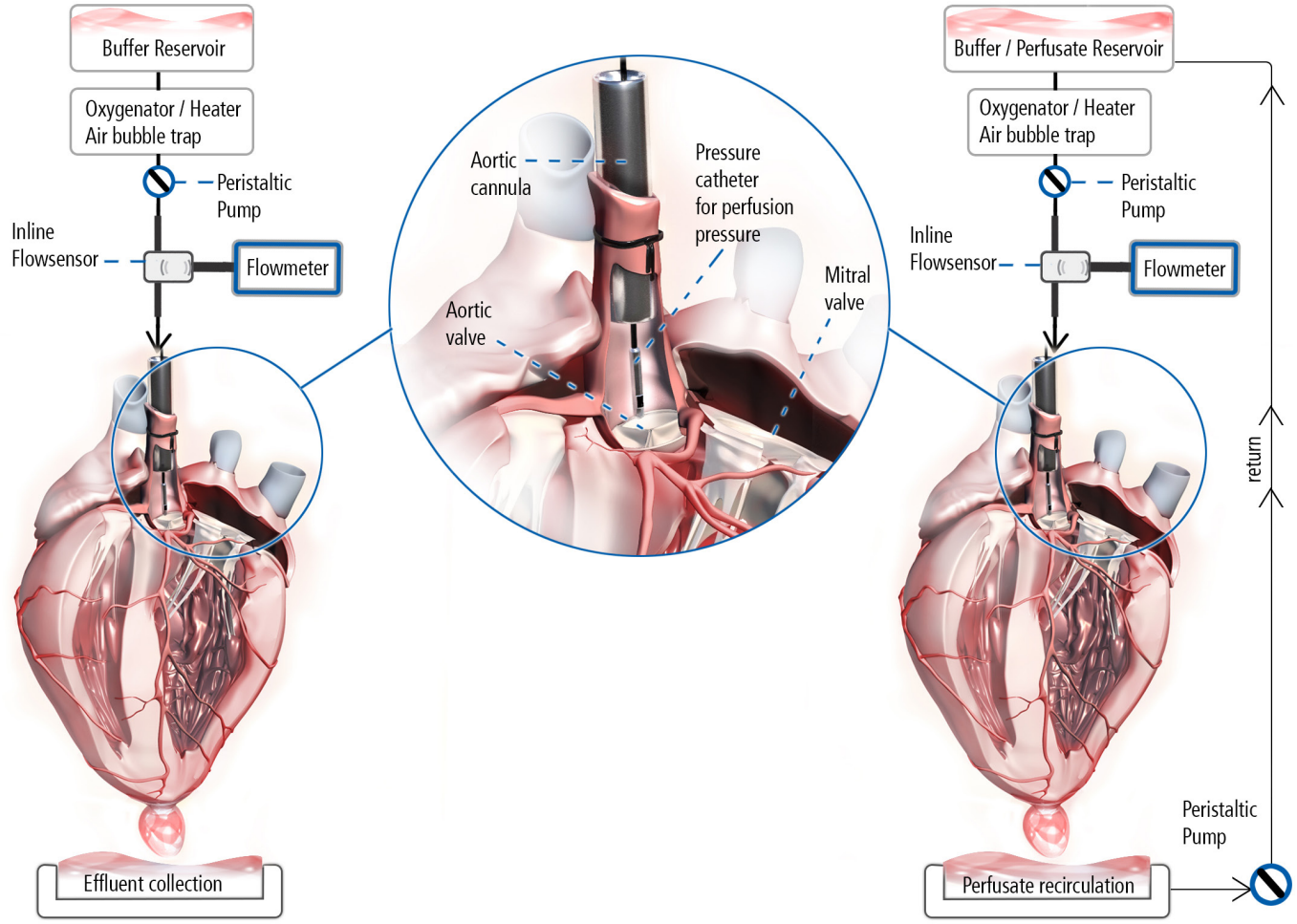
Drawing on the left shows buffer reservoir and collection of coronary flow as effluate drains from coronary sinus and pulmonary artery. On the right the effluate is recirculated using a peristaltic pump (e.g. drug compound testing application). The inline volume flow sensor helps to determine the LV 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

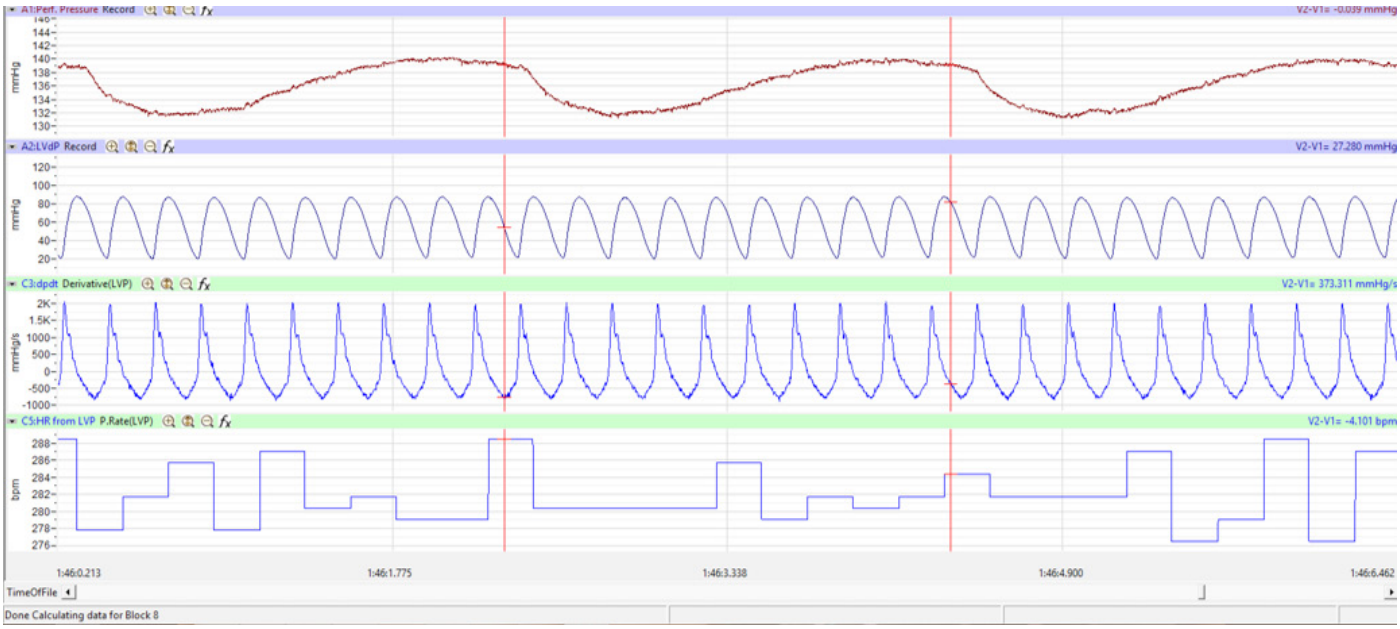
Both perfusion systems, when using non-blood perfusate, have coronary flow rates higher than in-vivo (8-12 ml/min/g of heart) (3). The constant flow system may override the auto-regulatory cardiac mechanisms otherwise guarded by pressure development in the system. Additionally, constant low flow rates 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.

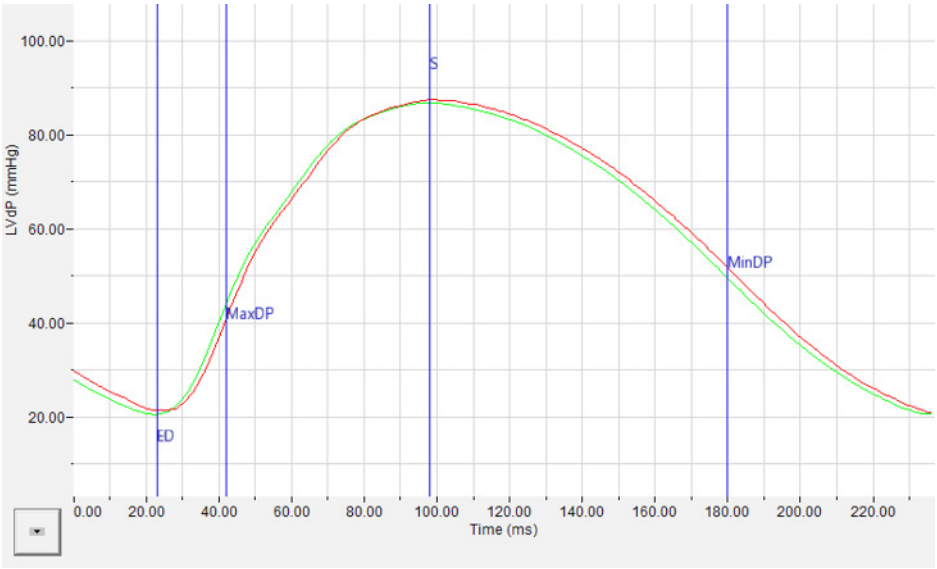


Basic set up of constant flow Langendorff. On the left, a peristaltic pump after the oxygenator/heater/ (bubble trap) provides constant flow. Inline flow sensor can be added to enable measuring the flow rate of buffer or perfusate/effluate. Depicted on the right, effluate is collected as it drains from 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.

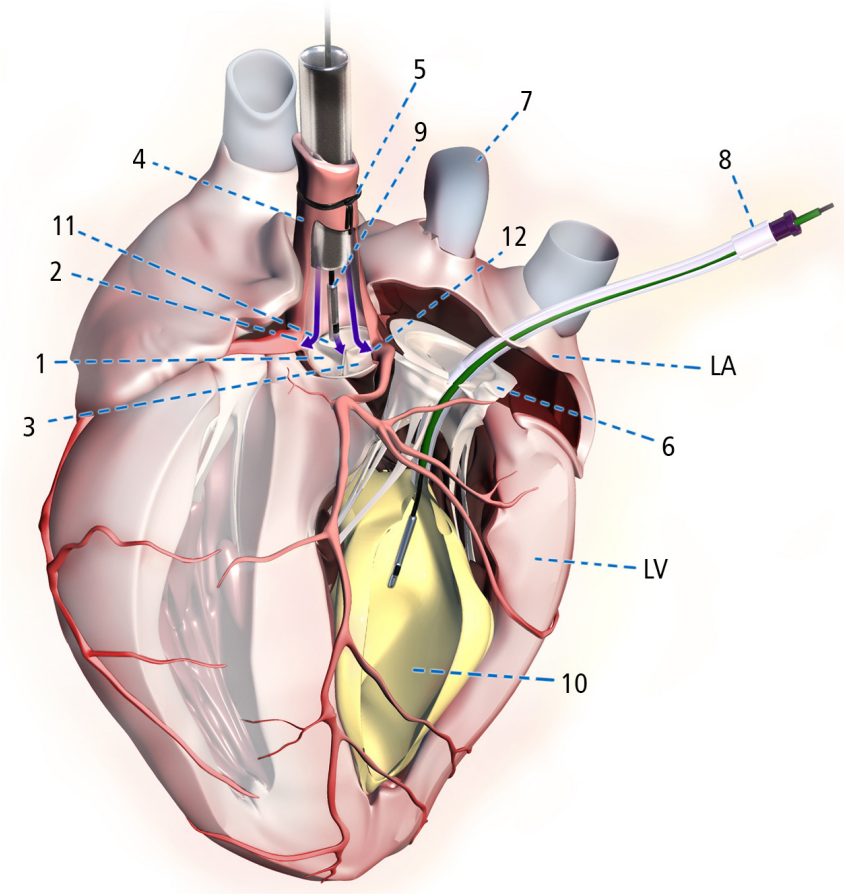


(Right) Data from the rat constant flow Langendorff system configuration LVdp trace with end diastole location (ED); dp/dt max (MaxDP); peak systolic pressure (S); and dp/dt min (MinDP) displayed.

	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.

REFERENCES

(1) Louch WE, Sheehan KA, Wolska BM. Methods in cardiomyocyte isolation, culture, and gene transfer. J Mol Cell Cardiol. 2011 Sep;51(3):288-98.

(2) Kadipasaoglu KA, Bennink GW, Conger JL, Birovljev S, Sartori M, Clubb FJ Jr, Noda H, Ferguson JJ, Frazier OH. An ex vivo model for the reperfusion of explanted human hearts. Tex Heart Inst J. 1993;20(1):33-9.

(3) Sutherland FJ, Hearse DJ. The isolated blood and perfusion fluid perfused heart. Pharmacol Res. 2000 Jun;41(6):613-27.

(4) Murakami M, Ohba T, Xu F, Satoh E, Miyoshi I, Suzuki T, Takahashi Y, Takahashi E, Watanabe H, Ono K, Sasano H, Kasai N, Ito H, Iijima T. Modified sympathetic nerve system activity with overexpression of the voltage-dependent calcium channel beta3 subunit. J Biol Chem. 2008 Sep 5;283(36):24554-60.

(5) Shibutani S, Osanai T, Ashitate T, Sagara S, Izumiyama K, Yamamoto Y, Hanada K, Echizen T, Tomita H, Fujita T, Miwa T, Matsubara H, Homma Y, Okumura K. Coronary vasospasm induced in transgenic mouse with increased phospholipase C-δ1 activity. Circulation. 2012 Feb 28;125(8):1027-36

(6) Xiao CY, Yuhki K, Hara A, Fujino T, Kuriyama S, Yamada T, Takayama K, Takahata O, Karibe H, Taniguchi T, Narumiya S, Ushikubi F. Prostaglandin E2 protects the heart from ischemia-reperfusion injury via its receptor subtype EP4. Circulation. 2004 May 25;109(20):2462-8.

(7) Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, Gooch R, Foley J, Newman J, Mao L, Rockman HA, Hammock BD, Murphy E, Zeldin DC. Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K+ channels and p42/p44 MAPK pathway. Circ Res. 2004 Sep 3;95(5):506-14.

(8) Döring HJ, Dehnert H, The Isolated Perfused Heart According to Langendorff, English edition, Biomess- technick-Verlag, West Germany, 1988.

(9) Reichelt ME, Willems L, Hack BA, Peart JN, Headrick JP. Cardiac and coronary function in the Langendorff-perfused mouse heart model. Exp Physiol. 2009 Jan;94(1):54-70.

(10) Ferdinandy P, Panas D, Schulz R. Peroxynitrite contributes to spontaneous loss of cardiac efficiency in isolated working rat hearts. Am J Physiol. 1999 Jun;276(6 Pt 2):H1861-7.

(11) Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion. J Mol Cell Cardiol. 2011 Jun;50(6):940-50.

(12) Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212: 804– 814, 1967.

(13) Liao R, Podesser BK, Lim CC. The continuing evolution of the Langendorff and ejecting murine heart: new advances in cardiac phenotyping. Am J Physiol Heart Circ Physiol. 2012 Jul 15;303(2):H156-67.

(14) Figueredo VM, Brandes R, Weiner MW, Massie BM, Camacho SA. Cardiac contractile dysfunction during mild coronary flow reductions is due to an altered calcium-pressure relationship in rat hearts. J Clin Invest. 1992 Nov;90(5):1794-802.

(15) Henderson KA, Borders RB, Ross JB, Huwar TB, Travis CO, Wood BJ, Ma ZJ, Hong SP, Vinci TM, Roche BM. Effects of tyrosine kinase inhibitors on rat isolated heart function and protein biomarkers indicative of toxicity. J Pharmacol Toxicol Methods. 2013 Jul-Aug;68(1):150-9.

(16) Hampton TG, Amende I, Travers KE, Morgan JP. Intracellular calcium dynamics in mouse model of myocardial stunning. Am J Physiol. 1998 May;274(5 Pt 2):H1821-7.

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THE MEASURE OF BETTER RESULTS.

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.

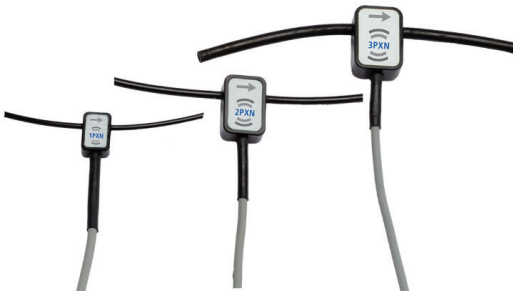
LEFT ATRIAL CANNULATION

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.

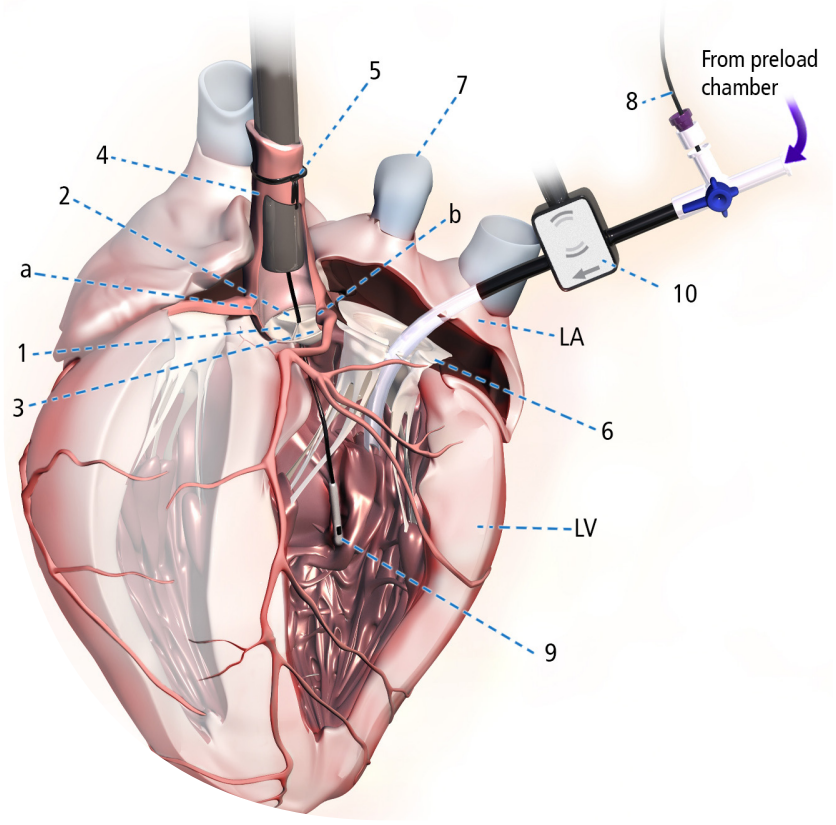
Working Heart Model for Cardiac Assessment Cont.

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)**

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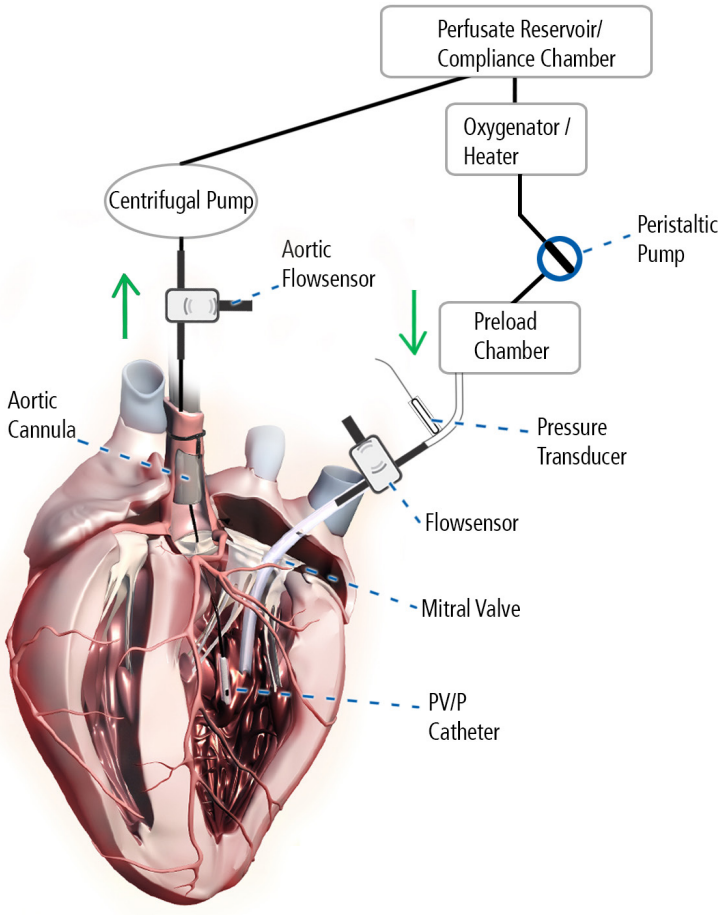
Working Heart Model for Cardiac Assessment Cont.

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.

Surgical Protocols

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THE MEASURE OF BETTER RESULTS.

Mouse Left Ventricle PV Measurement (Open Chest)

APPLICATION BASICS

Site:	Left Ventricle - Open Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure Volume
Catalog #:	FTH-1212B-3518, FTH-1212B-4018, FTH-1212B-4518

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

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 (towards the mouse's head) (Fig 3). Cut through the diaphragm (Fig 4) to expose the heart apex (Fig 5). Try to avoid any incisions around sternum to limit bleeding. 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).

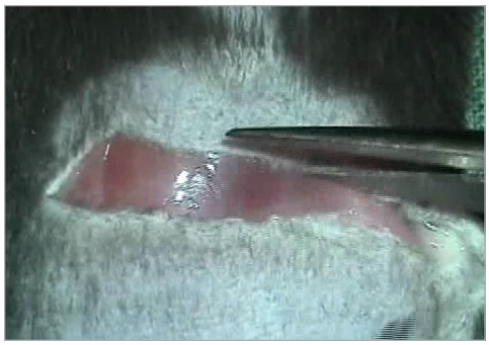

Fig. 1: Initial incision in the upper abdomen

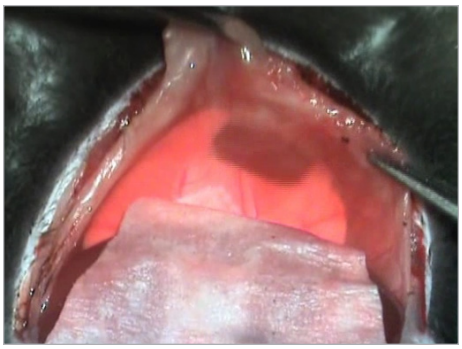

Fig. 2: Opening the abdomen wall

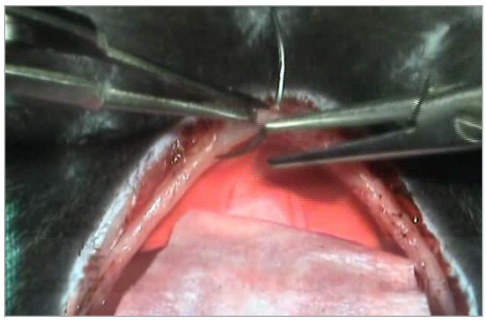

Fig. 3: Use a suture to hold the xiphoid in place

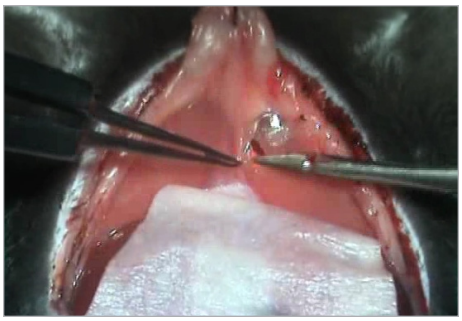

Fig. 4: Cut through the diaphragm

Use the 27 gauge needle for the LV apical stab (Fig 7). After successful stab, blood is found in the needle tip. As needle is withdrawn from the LV myocardium with your hand or with forceps covered by PE tubing, insert the 1.2F Catheter through the stab wound (Fig 8) until the distal electrode of the catheter is fully surrounded by

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Mouse Left Ventricle PV Measurement (Open Chest) Cont.

Surgical Approach Cont.

LV muscle (Fig 9). This is a critical step, for all electrodes have to be fully submerged in the ventricle's cavity. Position the Catheter to control for phase angle (ɵ) and admittance magnitude (Y) and collect pressure-volume (PV) signal in the form of a PV loop tracing.

Allow Catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol. Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should have a sinusoidal wave profile. If the PV Catheter lies in an off-center position, the phase signal may be distorted (signals will be relatively high with a low amplitude). Reposition the Catheter until a more central position is found, where magnitude waves are at their largest and phase waves are stable and devoid of noise or spikes. See "Proper PV Catheter Placement in the Left Ventricle" on page 45 for more details. Once optimal Catheter position is obtained, perform a "baseline scan" on the ADV500/ADVantage control unit - end-systolic and end-diastolic blood conductance ($G_{b\text{-}ED}$ and $G_{b\text{-}ES}$) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan, as necessary, throughout the experiment to ensure most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

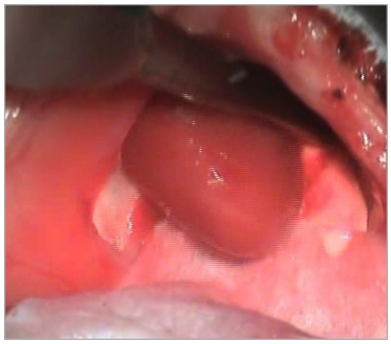

Fig. 5: Expose the heart apex

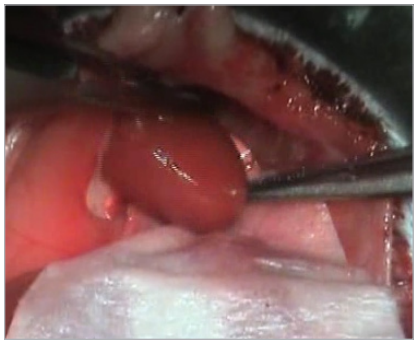

Fig. 6: Open the pericardium

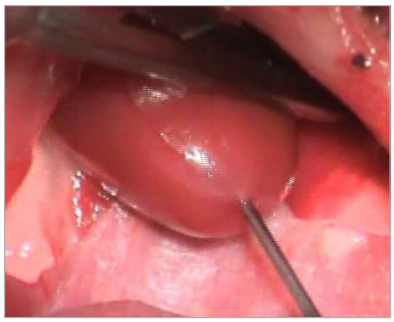

Fig. 7: Stab apex with 27G needle

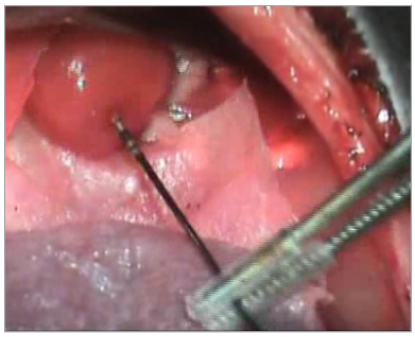

Fig. 8: Carefully insert Catheter into stab wound

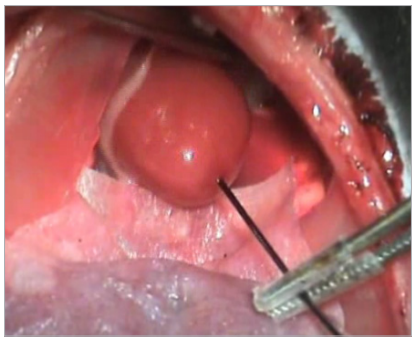

Fig. 9: Submerge all electrodes

IVC occlusion is used to derive various load-independent indices of cardiac function. Abdomen is opened and (5-0 silk) suture is placed under the vena cava, carefully separated from adventicia and thoracic aorta, above the liver at close proximity of the heart. This position will ensure an immediate drop of blood volume to better control and compare data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel. Shut off the ventilation for a few seconds prior to and during occlusion to acquire data without lung motion artifacts.

At the end of the experiment, carefully remove the PV 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, see "Cleaning Guidelines for Catheters" on page 53.

Mouse Left Ventricle PV Measurement (Closed Chest)

APPLICATION BASICS

Site:	Left Ventricle - Closed Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure Volume
Catalog #:	FTH-1212B-3518, FTH-1212B-4018, FTH-1212B-4518

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Note: Preforming an IVC occlusion will require a second incision in the abdomen of the mouse.

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.

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 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. 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).

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 mouse'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.

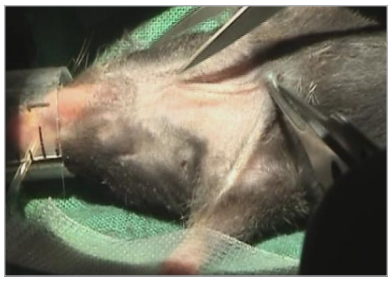

Fig. 1: Initial incision under the chin

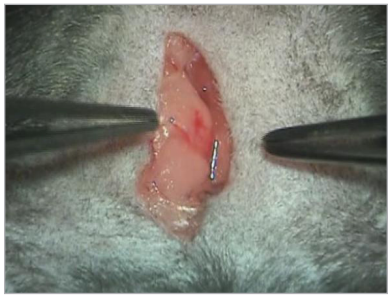

Fig. 2: Dissect glandular tissue from skin

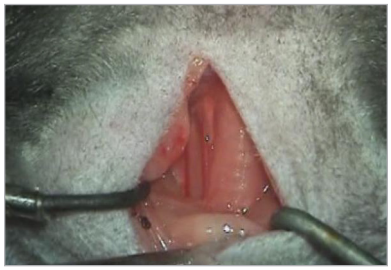

Fig. 3: Retract skin to expose site

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Mouse Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

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 retracting it towards the head. Place 2nd suture (Fig 7) and retract it 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. Slide 3rd suture under the segment but do not tie it off (Fig 7). This suture will be tied off when PV 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 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 require more time to successfully introduce the Catheter, an introducer might allow more time to locate the insertion site in the collapsed RCA, limiting blood loss on catheterization. When completely satisfied with the RCA opening, carefully proceed (Fig 10) and lift the sternal clamp and insert 1.2F tetrapolar pressure-volume Catheter into the opening by passing both sets of volume electrodes. Position and tie off the first suture around the Catheter past the second set of rings (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).

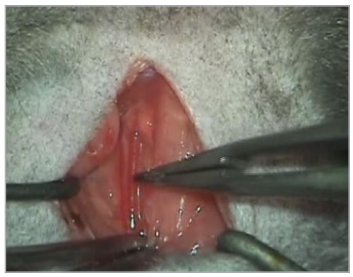

Fig. 4: Expose & dissect RCA

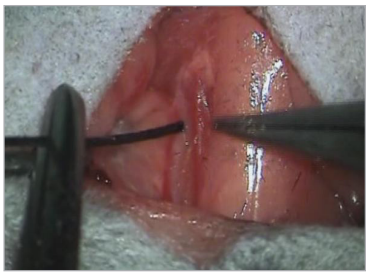

Fig. 5: Use hemostat to draw suture under the RCA

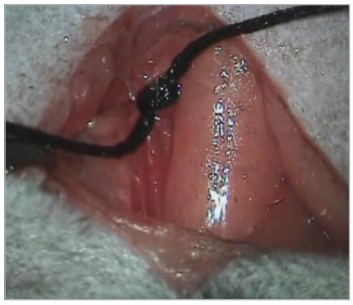

Fig. 6: Tie suture to proximal end of RCA

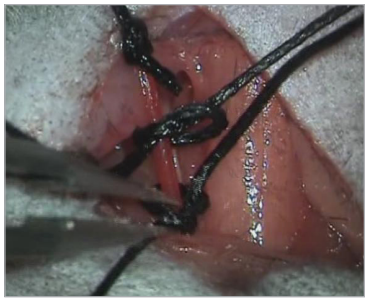

Fig. 7: Three sutures around the RCA

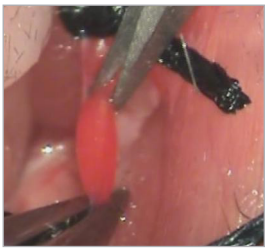

Fig. 8: Carefully cut RCA

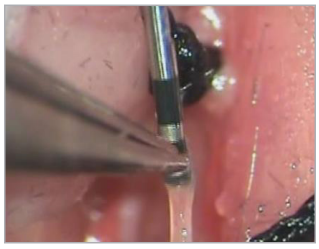

Fig. 9: Carefully insert Catheter

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Mouse Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

With the Catheter in the RCA, get a feel for the degree of resistance by 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 it slipping 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 (please see "Optimizing Catheter Life Span" on page 56). Position the Catheter to control for phase angle (ɵ) and admittance magnitude (Y) and collect pressure-volume (PV) signal.

Allow Catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol. Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should have a sinusoidal wave profile. If the PV Catheter lies in an off-center position the phase signal may be distorted (signals will be relatively high with a low amplitude). See "Proper PV Catheter Placement in the Left Ventricle" on page 45 for more details. Reposition the Catheter until a more central position is found, where magnitude waves are at their greatest and phase waves are stable and devoid of noise or spikes. Once the optimal Catheter position is obtained, preform a "baseline scan" on the ADV500/ADVantage control unit. End-systolic and end-diastolic blood conductance ($G_{b\text{-}ED}$ and $G_{b\text{-}ES}$) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure the most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

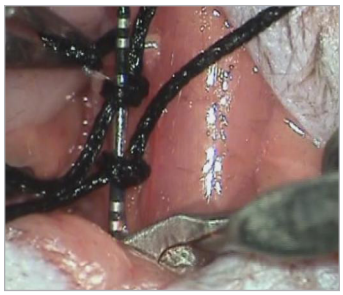


Fig. 10: Proceed with Catheter insertion

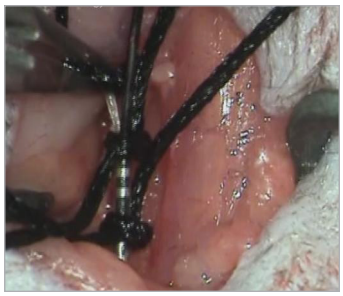


Fig. 11: Fully insert both sets of volume rings past the sutures

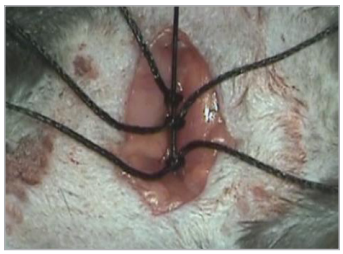


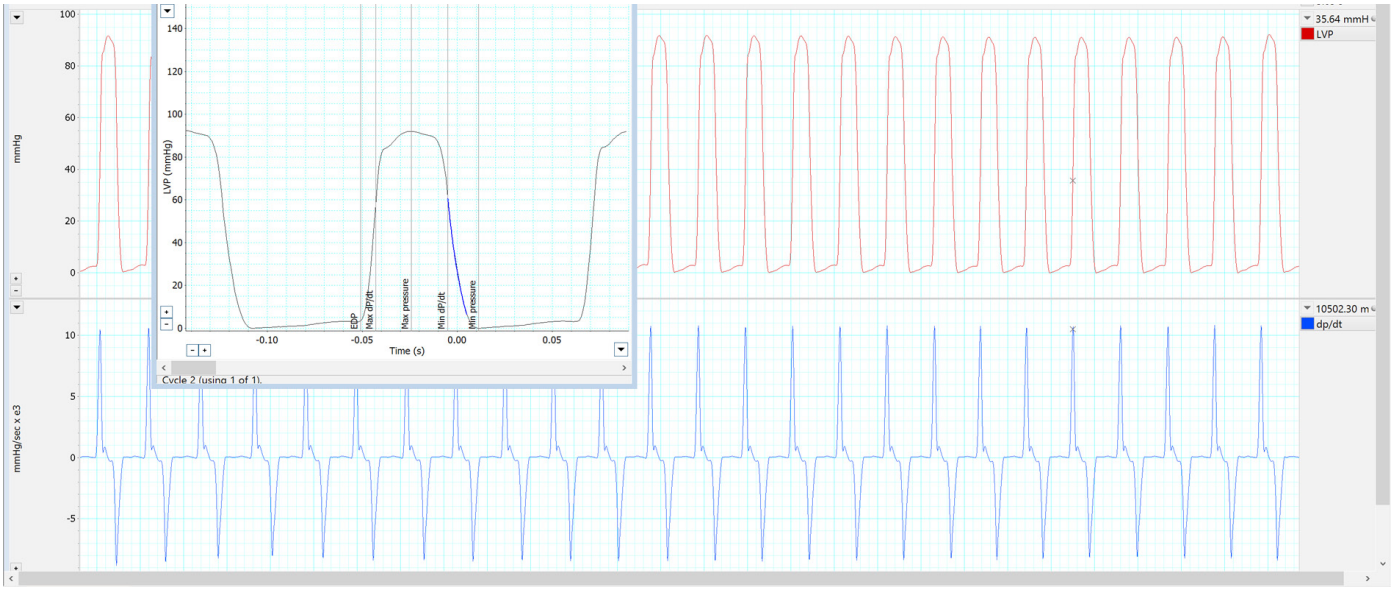
Fig. 12: Secure Catheter in place

IVC OCCLUSION

IVC occlusion is used to derive various load-independent indices of cardiac function. In order to preform an IVC occlusion, a second surgical incision must be made in the abdomen to expose the vena cava. Carefully separate the vena cava from adventicia and thoracic aorta, above the liver close to the heart. The best technique is to place a 5-0 silk suture around the vena cava located as close as possible to heart. This position will ensure an immediate drop of blood volume to better control and compare data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel. Shut off the ventilation for a few seconds prior to and during occlusion to acquire data without lung motion artifacts.

At the end of the experiment, carefully remove the PV Catheter by gently pulling it back through the RCA. 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, see "Cleaning Guidelines for Catheters" on page 53.

Mouse Left Ventricle PV 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	PMAX mmHg	PMIN mmHg	EDP mmHg	MEAN PRESSURE mmHg	SYSTOLIC DURATION sec	DIASTOLIC DURATION sec	CYCLE DURATION sec	dPMAX mmHg/sec	dPMIN 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 PV Measurement (Closed Chest)

APPLICATION BASICS

Site:	Right Ventricle - Closed Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure Volume
Catalog #:	FTH-1212B-3518, FTH-1212B-4018, FTH-1212B-4518

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Note: Preforming an IVC occlusion will require a second incision in the abdomen of the mouse.

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.

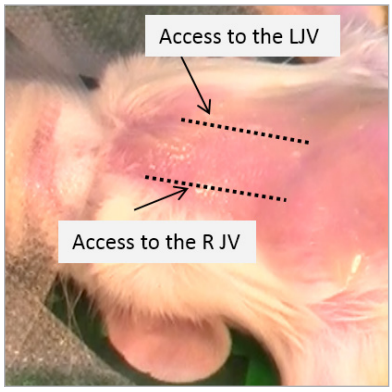


Fig. 1: Location of access to the RJV

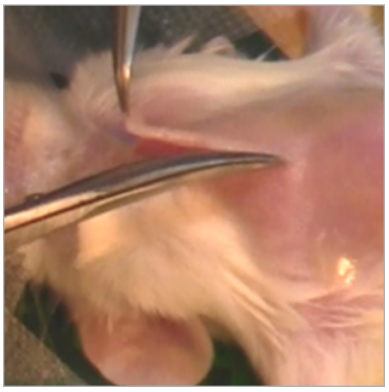


Fig. 2: Initial incision under the chin

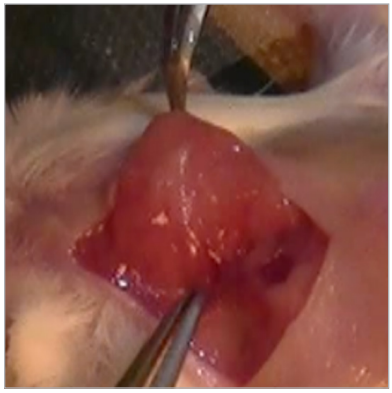


Fig. 3: Retract skin to expose site

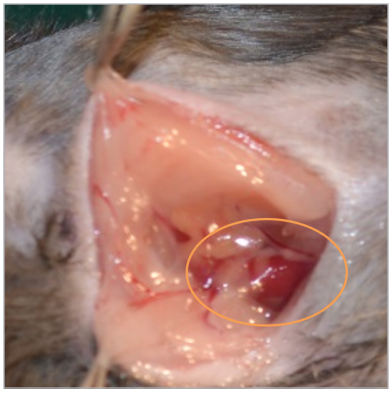


Fig. 4: Expose & dissect RJV

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

Mouse RV PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

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 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 an 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 closed. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. Keep in mind 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).

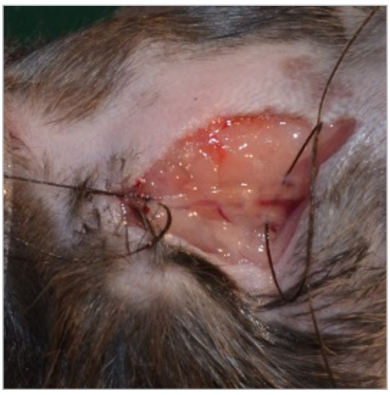


Fig. 5: Tie suture to the cranial end of the exposed RJV. Loosely prepare suture at the caudal end.

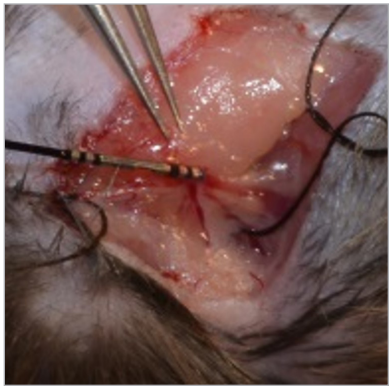


Fig. 6: Position Catheter parallel to the RJV

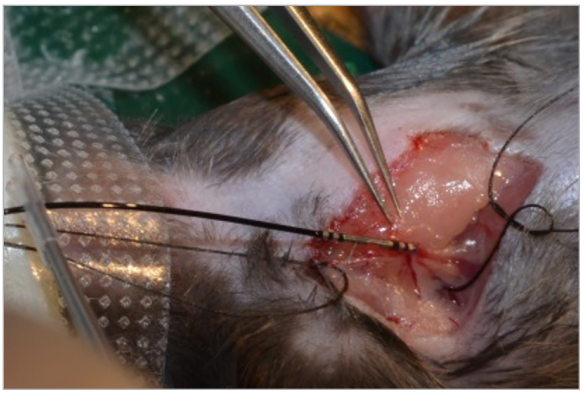


Fig. 7: Carefully insert the Catheter into the RJV

CATHETER INTRODUCTION AND POSITIONING

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

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, passing both sets of volume electrodes. Position and tie off the caudal suture around the catheter passed the second set of rings. 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.

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 RV PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

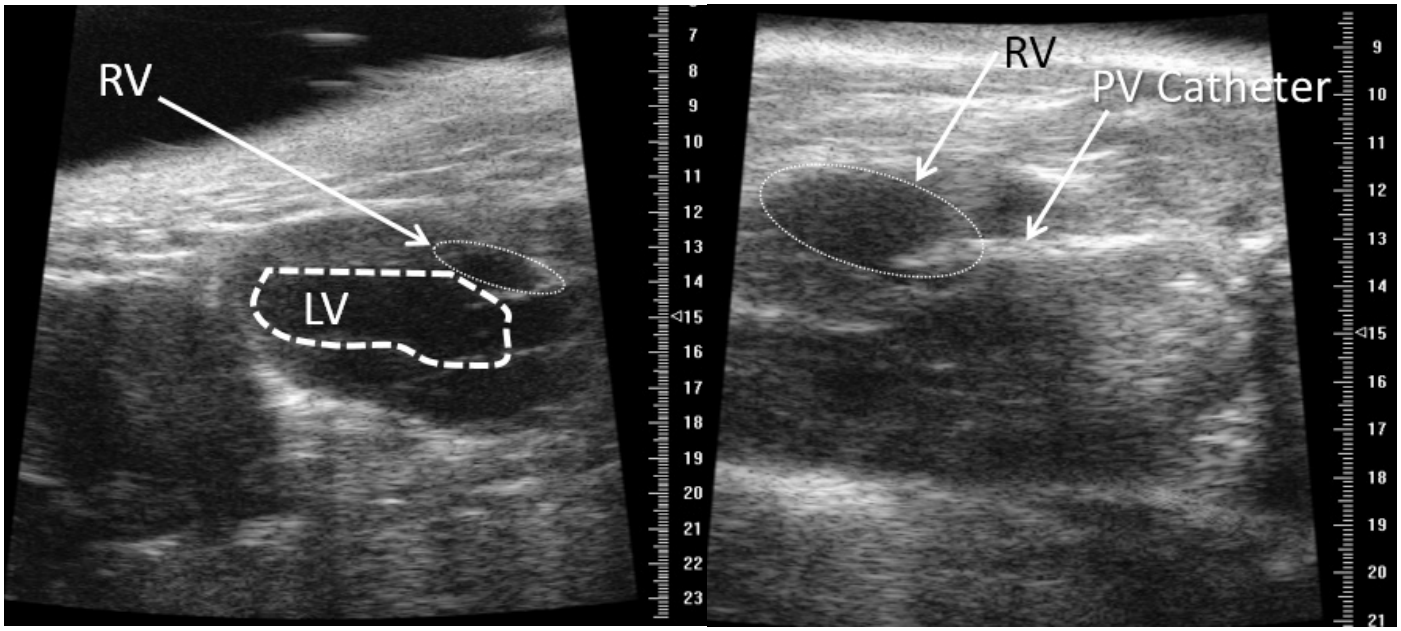


Fig. 8: (a) Long axis echocardiography image. LV and RV are encircled (b) The scan head is repositioned to better visualize the RV for catheter insertion.

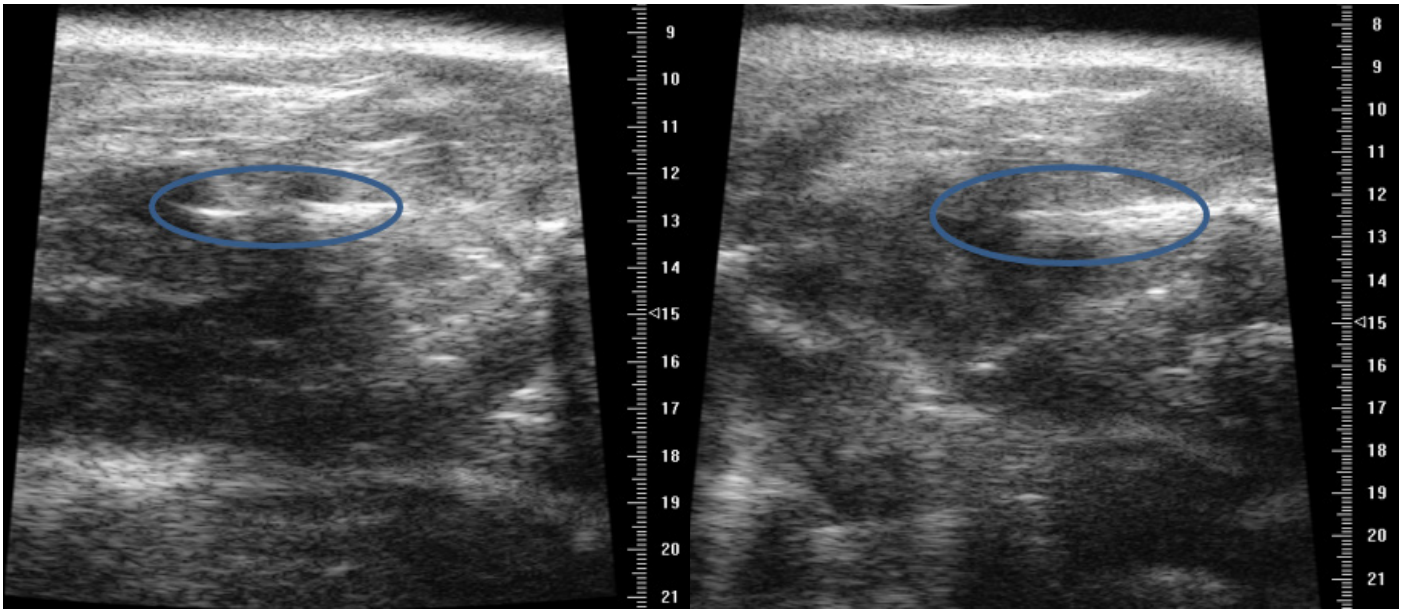


Fig. 9a: The blue circle shows the tip of the PV catheter advancing into position in the middle of the RV (seen at 2D plane)

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

Mouse RV PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

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 (not shown, optional, as 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.

For closed chest preparation it is necessary to use echocardiography guidance to successfully position the PV 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 PV measurements (Fig 10A ,10B). When the catheter is in a good position, you will find good RV pressure signal (Fig 11). Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should measure sinusoid wave signals. In case of off-center position, acquired sinusoid signals might be distorted (low amplitude, frequency etc.). Reposition Catheter until an optimal position is found – essentially this is where magnitude waves are at their largest and phase waves are stable and devoid of noise or spikes. The researcher should also view Pressure and Magnitude in an XY plane to assist in their search for optimal Catheter position.

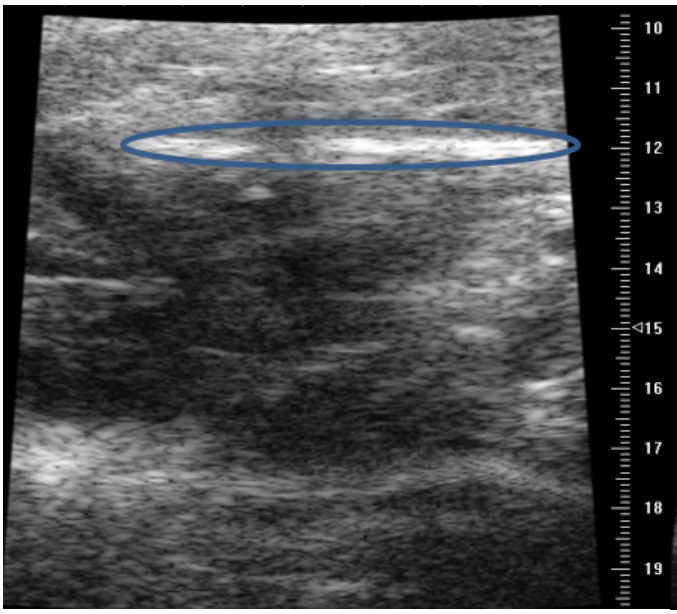


Fig. 10a: Catheter touching to the septum (blue circle)and as described it is also too far in the RV (long axis view)

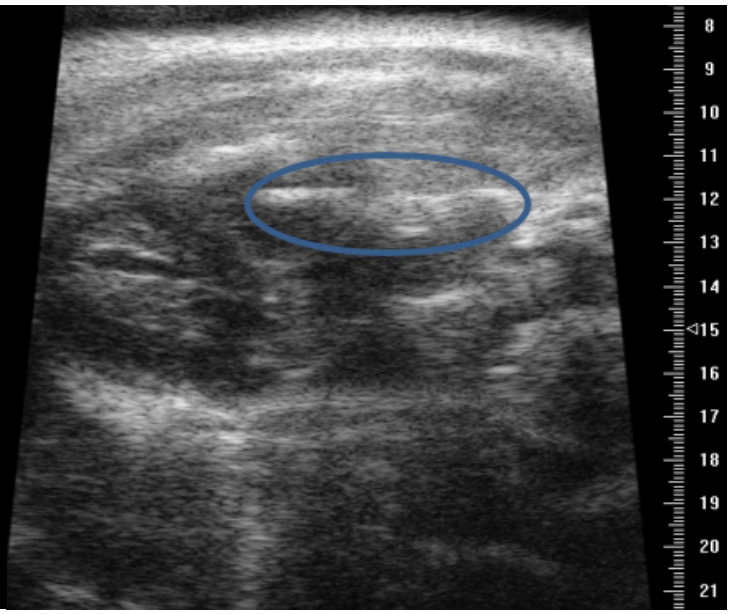


Fig. 10b: Catheter pulled back into better position. Both RV free wall and septum are away from the catheter at this point .This is a very good position for pressure-volume recording in the RV. Also check Phase angle for the amplitude and range before data recording

Mouse RV PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

Allow the Catheter to stabilize in the RV for 5-10 min before marking the data file to start protocol. Once optimal Catheter position is obtained, preform a "baseline scan" on the ADV500/ADVantage control unit. End-systolic and end-diastolic blood conductance (Gbed and Gbes) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

IVC OCCLUSION

IVC occlusion is used to derive various load-independent indices of cardiac function. In order to preform an IVC occlusion, a second surgical incision must be made in the abdomen to expose the vena cava. Carefully separate the vena cava from adventitia and thoracic aorta, above the liver at close proximity to the heart. The best technique is to place a 5-0 silk suture around the vena cava located as close as possible to heart. This position will ensure an immediate drop of blood volume to better control and compare data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel. Shut off the ventilation for a few seconds prior to and during occlusion to acquire data without lung motion artifacts.

At the end of the experiment, carefully remove the PV 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).

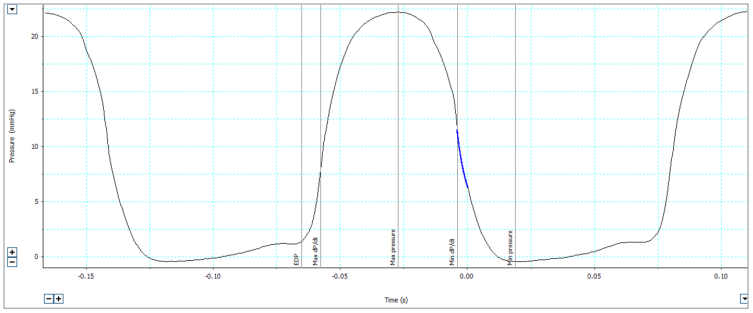


Fig. 11: Pressure signal from the mouse right ventricle.

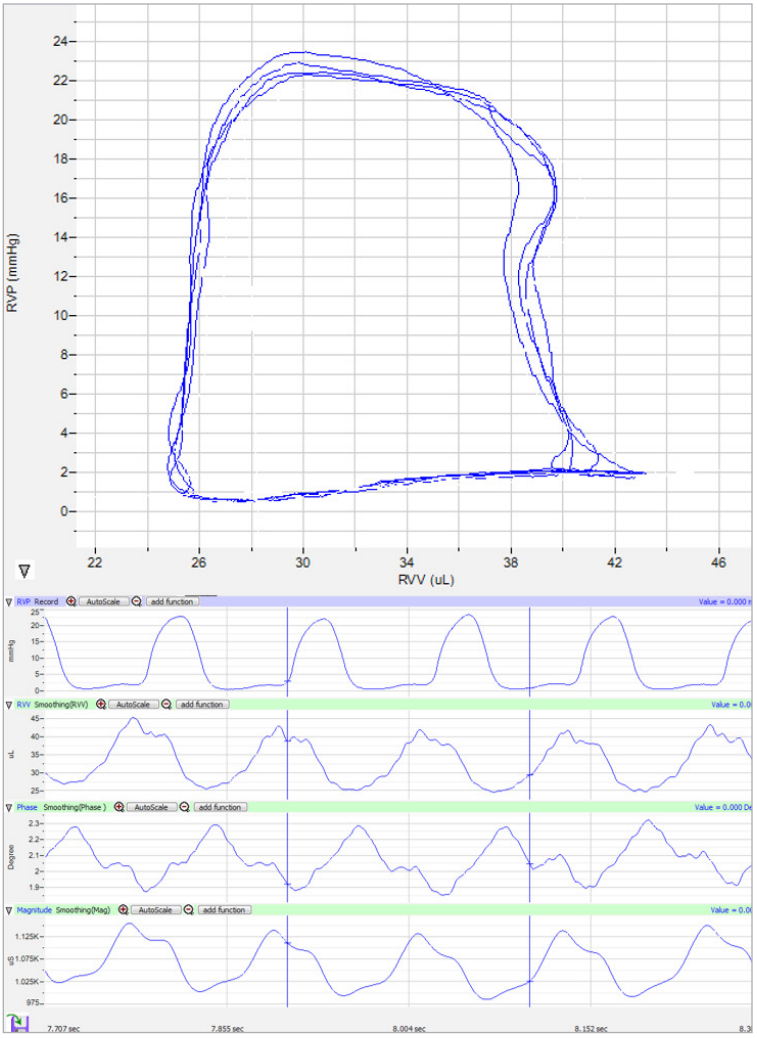


Fig. 12: PV loops and pressure, magnitude, phase and volume data from the mouse right ventricle.

Rat Left Ventricle PV Measurement (Open Chest)

APPLICATION BASICS

Site:	Left Ventricle - Open Chest
Species:	Rat
Body Weight:	200 - 500 grams
Duration:	Acute

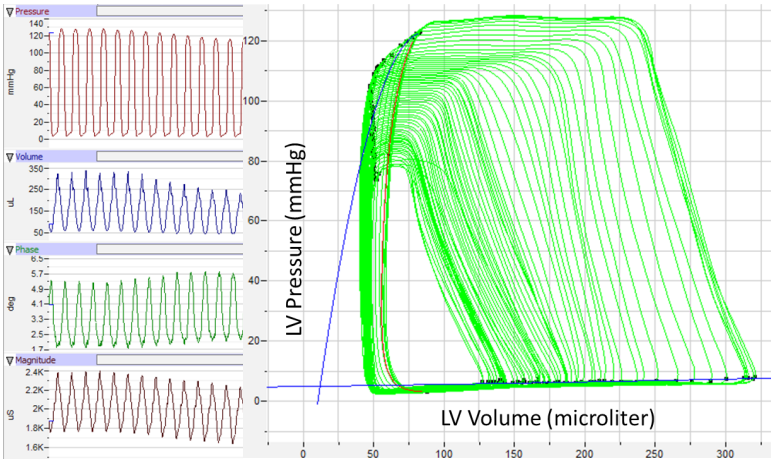
CATHETER

Size:	1.9F
Type:	Pressure Volume or VSL Pressure Volume
Catalog #:	FTH-1912B or FTH-1918B

SYSTEM

ADV500 / ADVantage

PV DATA AND LOOPS



Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Surgical Approach

Prior to surgery, soak the tip of the PV Catheter in 0.9% saline for ~ 20 minutes. After soaking, adjust the pressure balance to zero for atmospheric pressure. See “Balancing Pressure Sensors Before Use” on page 39 for guidelines.

Anesthetize rats with 3- 4% Isoflurane and maintain anesthesia with 2% Isoflurane by ventilator. Secure animal in dorsal position on the heating pad. Make V shape 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). Cut through the diaphragm to expose the heart apex (Fig. 3). Try to avoid any incisions around sternum to limit bleeding. Try not to artificially retract rib cage. Gently maneuver the apex, using Q-tips into the diaphragm opening.

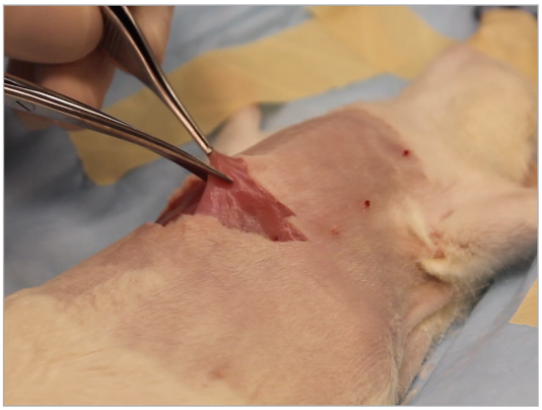


Fig. 1: Initial incision in the upper abdomen of rat in dorsal position

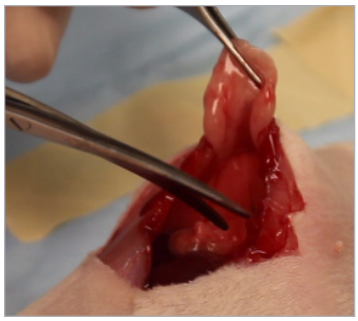


Fig. 2: Open the abdominal wall

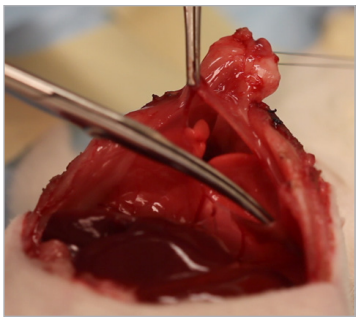


Fig. 3: Cut through the diaphragm to expose the heart apex

ACKNOWLEDGMENTS

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

REFERENCES

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 2012. 94, 492-500

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Rat Left Ventricle PV Measurement (Open Chest) Cont.

Surgical Approach Cont.

Use the 25G needle for the LV apical stab. After a successful stab, blood is found in the needle tip (Fig. 4). As the needle is withdrawn from the LV myocardium, with your other hand, insert the 1.9F Catheter through the stab wound (Fig. 5) until the distal electrode of the catheter is fully surrounded by LV muscle (Fig. 6). This is a critical step where all electrodes have to be fully submerged in the ventricle's cavity. Position the catheter to control for phase angle (ɵ) and admittance magnitude (Y) and collect pressure-volume (PV) signal.

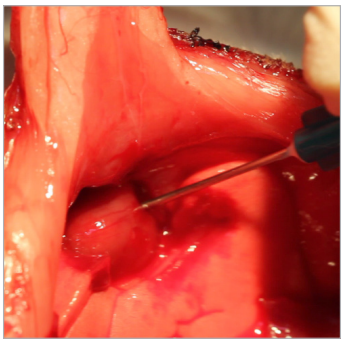

Fig. 4: Stab apex with a 25G needle

Allow the Catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol. Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should measure sinusoid wave signal. In the case of an off-center position, acquired sinusoid signals might be distorted as e.g. (low amplitude, frequency etc.). See "Proper PV Catheter Placement in the Left Ventricle" on page 45 for more details. Reposition the Catheter until an optimal position is found – essentially this is where magnitude waves are at their largest and phase waves are stable and devoid of noise or spikes. The researcher should also view Pressure and Magnitude in an XY plane to assist in their search for optimal Catheter position. Once optimal Catheter position is obtained, perform a "baseline scan" on the ADV500/ADVantage control unit - end-systolic and end-diastolic blood conductance ($G_{b\text{-}ED}$ and $G_{b\text{-}ES}$) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure the most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

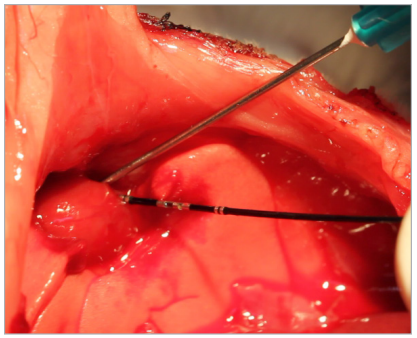

Fig. 5: Insert Catheter into the stab wound.

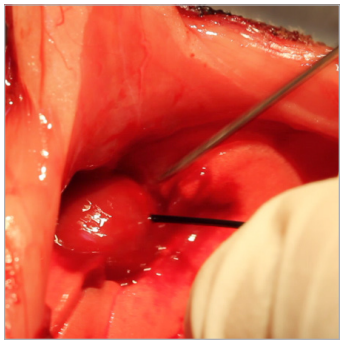

Fig. 6: Submerge all electrodes

IVC occlusion is used to derive various load-independent indices of systolic function. During preparation of the open chest, an IVC occlusion suture (5-0 silk) is placed under the vena cava as it is carefully separated from its adventicia and thoracic aorta (Fig. 7). 5-0 silk is placed above the liver at close proximity of the heart. This position will ensure an immediate volume drop to better control and compare the data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel (Fig. 8). Shut off the ventilation for a few seconds to acquire data without lung motion artifacts.

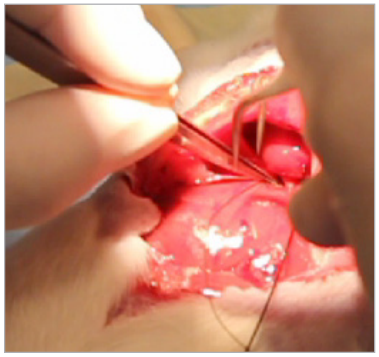

Fig. 7: Place sutures for IVC occlusion

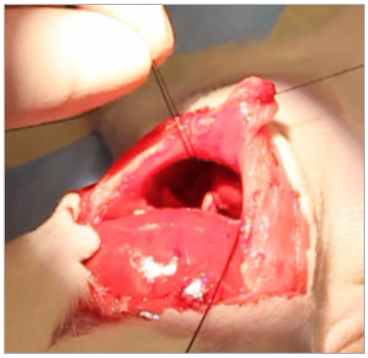

Fig. 8: Pull sutures to perform IVC occlusion

At the end of the experiment, carefully remove the PV 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 see "Cleaning Guidelines for Scisense Catheters" on page 53.

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Rat Left Ventricle PV Measurement (Closed Chest)

APPLICATION BASICS

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

CATHETER

Size:	1.9F
Type:	Pressure Volume or VSL Pressure Volume
Catalog #:	FTH-1912B or FTH-1918B

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Note: Performing an IVC occlusion will require a second incision in the abdomen of the rat.

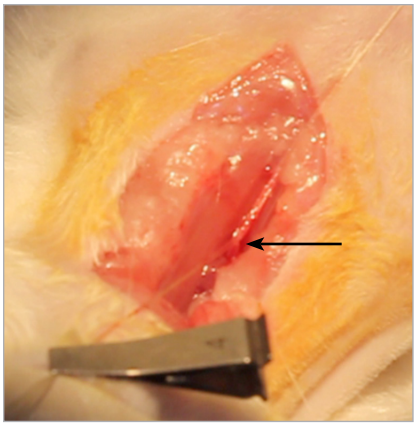

Fig. 1: Isolated RCA with sutures knotted around artery

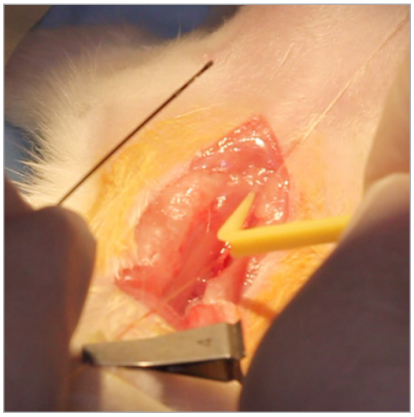

Fig. 2: Vascular introducer (yellow) is used to open the RCA in preparation for Catheter insertion

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). Remember to avoid pressure on these muscles to allow the rat 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 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-sympathatic 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.

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Rat Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

At this stage, 5-0 sutures can be placed around the 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. Retract it towards the head (Fig. 1). At this point, the RCA has been retracted proximally and distally. The 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.

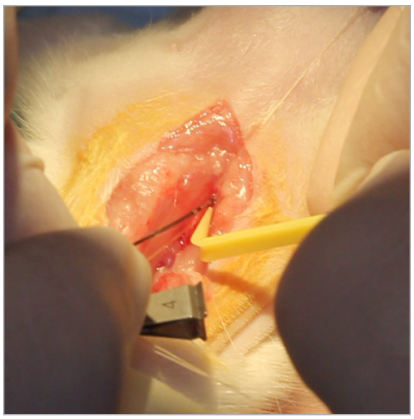


Fig. 3: Carefully remove the introducer and insert the Catheter

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.

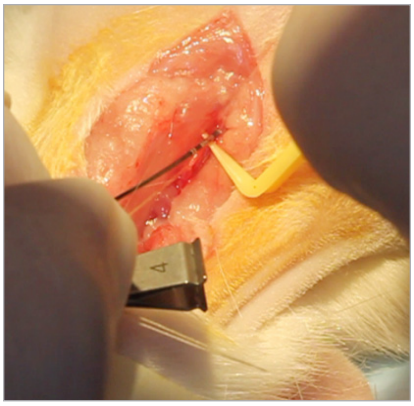


Fig. 4: Use the introducer to help Catheter insertion

When completely satisfied with the RCA opening, carefully proceed to insert the tetrapolar pressure-volume micro-manometer 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 "Optimizing Catheter Life Span" on page 56 for best practices). 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 the 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 past the second set of rings. Ideally, there should be little 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 it slipping out. Slide the Catheter slowly towards the heart. Position the Catheter to control for phase angle (ɵ) and admittance magnitude (Y). Both signals should measure sinusoid wave signal. In case of off-center position, acquired sinusoid signals might be distorted (low amplitude, frequency etc.). See "Proper PV Catheter Placement in the Left Ventricle" on page 45 for more information.

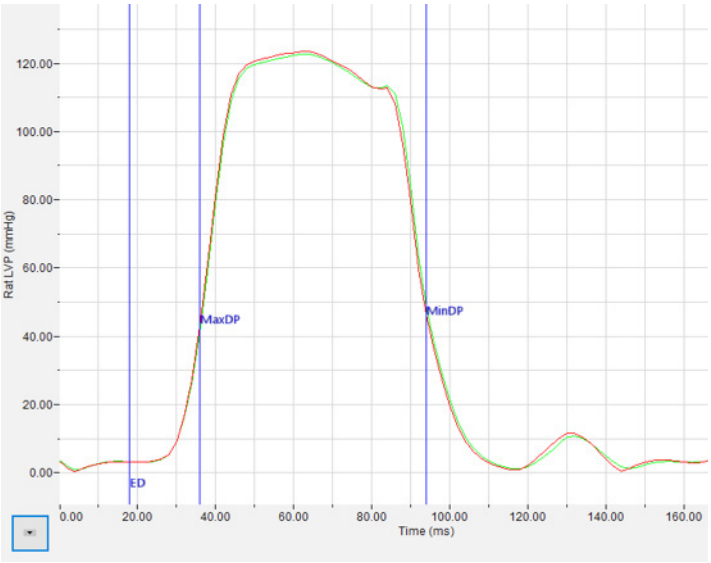
Allow Catheter to stabilize in the LV for 5-10 min before starting the protocol. Once optimal Catheter position is obtained, preform a "baseline scan" on the ADV500/ADVantage control unit - end-systolic and end-diastolic blood conductance ($G_{b\text{-}ED}$ and $G_{b\text{-}ES}$) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion. Every time an adjustment is performed (Catheter position, ventilation, temperature etc.) re-record baseline PV.

Rat Left Ventricle PV Measurement (Closed Chest) Cont.

IVC OCCLUSION

IVC occlusion is used to derive various load-independent indices of cardiac function. In order to perform an IVC occlusion, a second surgical incision must be made in the abdomen to expose the vena cava. Carefully separate the vena cava from its adventicia and thoracic aorta, above the liver close to the heart. The best technique is to place a 5-0 silk suture around the vena cava as close as possible to heart. This position will ensure an immediate volume drop to better control and compare the data sets. IVC occlusion is performed by pulling upward on 5-0 suture. Shut off the ventilation for a few seconds prior to and during occlusion to acquire data without lung motion artifacts.

At the end of the experiment, carefully remove the PV Catheter by gently pulling it back through the RCA. Immediately, insert Catheter tip into 5 ml saline pre-filled syringe. Clean Catheter as soon as possible according to proper care guidelines to prolong the Catheter's life, see "Cleaning Guidelines for Catheters" on page 53.



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 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

ACKNOWLEDGMENTS

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

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 2012. 94, 492-500.

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Pig Left Ventricle PV Measurement (Closed Chest)

APPLICATION BASICS

Site:	Left Ventricle - Closed Chest, Right Femoral Artery or Right Carotid Artery Access
Species:	Pig (mini pig)
Body Weight:	20 - 70 kg
Duration:	Acute

CATHETER

Size:	5.0F or 7.0F
Type:	VSL Pressure Volume
Catalog #:	FDH-5018B-E(1, 2, 3 or 4)45(A or D) FDH-7018B-E(2, 3 or 4)45(A or D)

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion (balloon catheter occlusion) as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Anatomical Landmarks

Right femoral artery (RFA) is approached while the pig is placed in dorsal recumbence and the right rear leg is retracted laterally. RFA is located by palpating the area of inner thigh and pressure is applied to detect pulsation of the artery in the femoral canal.

The right carotid artery (RCA) passes cranially along the right side of the trachea near the larynx in the close proximity of vago-sympathetic trunk. In pigs, the RCA is located deeper, as compared to humans, which doesn't allow palpation of the arterial pulse. Major muscles (sternohyoid and sternomastoid) in the area might be moved aside to allow better ventral neck access. The RCA divides into: right external carotid artery to supply head and neck and the right internal carotid artery that passes through carotid canal into base of skull.

ENDOTRACHEAL (ET) INTUBATION

Endotracheal intubation ensures artificial control of respiration and protects the airways from aspiration of foreign material. When anesthetizing pigs for more than 1 hour, ET intubation is recommended. ET intubation is typically performed orotracheally after 10-15 min of gaseous anesthesia. Prepare Laryngoscope with long straight handle (250 mm), local anesthetic spray (Xylocaine endotracheal spray, 2% Lidocaine), syringe with air, tape for tracheotube (TT) fixation and slings to open the mouth. Allow the pig's head to be situated close to the end of the surgical table. V shaped stainless steel surgical table is one of best for head stabilization. Constantly aspirate saliva to limit salivation and use Atopine or Glycopyrrolate.

To introduce tracheotube (TT), open the mouth wide using slings and pull the tongue out. Introduce the laryngoscope to the vocal chords and spray both chords with Lidocaine. Withdraw the laryngoscope and about 5 min later re-introduce both the laryngoscope and tracheotube, pre-sprayed with xylocaine. The laryngoscope is passed into the pharyngeal cavity and depresses the tongue, exposing the epiglottis. The tip of the laryngoscope is then used to press the epiglottis upward towards the base of the tongue, revealing the laryngeal opening. Introduce TT into larynx through the vocal cords. TT is then advanced into the trachea during expiration. The pig will cough reflexively when TT is inserted, and might expel a large amount of air through TT. Inflate the balloon cuff with air, and connect TT to the anesthesia machine. TT is then fixed on the snout and taped. Any signs of cyanosis or gasping may indicate improper TT placement. If possible, shorten the anesthesia circuitry to limit dead space.

Insufficient anesthesia and unsuccessful repeats to insert TT may lead to laryngospasm. Resistance should not be felt on insertion. If you feel resistance on TT introduction, please remember that pigs have a right cranial bronchus located before the tracheal bifurcation and also some breeds have small deep pharyngeal diverticulum dorsal to the larynx that can start to bleed profusely when penetrated. Additionally, over-inflation of balloon cuff might cause swelling, edema and obstruction of airways.

Pig Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach: Artery and Vein Catheterization

FEMORAL PERCUTANEOUS APPROACH

To facilitate access to the femoral canal, retract the rear hooves caudally with slight flexion of the hip joint and restrain them. For a percutaneous approach, locate the right femoral artery (RFA) by using anatomy landmarks and/or pulse palpation in the femoral canal or with the help of ultrasound guidance (USG). Use large 18-gauge, 2 3/4-inch Seldinger needle or trocar to percutaneously introduce it into the arterial lumen while advancing round tipped 0.038-inch Seldinger guidewire through the needle lumen into the RFA (Fig. 1). Withdraw the trocar from the skin. Do not damage the Seldinger guidewire. If under USG, examine position of the wire in the vessel lumen. Thread the dilator cannula through the guidewire into the RFA and advance it to desired position. A small incision might be necessary to insert the introducer past the dilator. Remove the Seldinger guidewire and introducer, leaving a blunt 7-10F cannula with blood sampling port in the RFA. It is advisable to use an access port at least 2 french sizes bigger than the PV Catheter. The cannula is sutured to skin to prevent movement (Fig. 2).

The femoral vein is located in the same adventitia, just medial to the artery. Use a similar method, as for the femoral artery, for cannula insertion. Use the LFV to insert the balloon catheter. It is best to use an access port at least 2F sizes bigger than the balloon catheter.

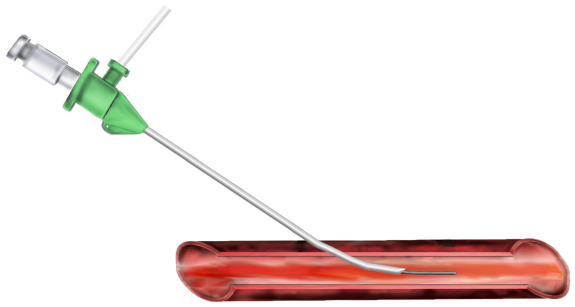

Fig. 1: Introduction of guidewire into the femoral artery using the Seldinger technique

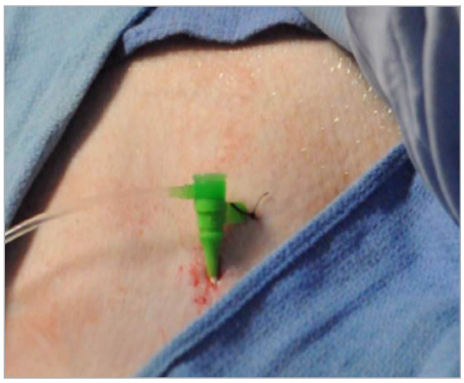

Fig. 2: Femoral vasculature access via percutaneous Seldinger technique

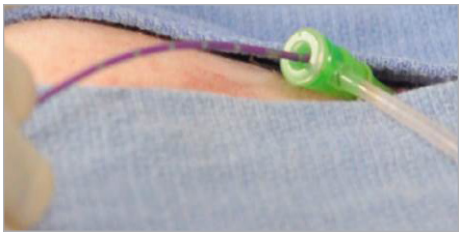

Fig. 3: Introduction of 5F pigtail pressure-volume admittance catheter into percutaneously catheterized right femoral artery (RFA)

FEMORAL CUT-DOWN APPROACH

The RFA and LFV are approached while the pig is placed in dorsal recumbence and the rear legs are retracted laterally. Pulsation of the superficial part of medial saphenous artery is identified in the skin fold between mm. gracilis and sartorius. Perform a skin incision cranial to this point using scalpel or electric cautery. Dissect the underlying subcutaneous tissue using blunt-tip scissors. Divide the fascia of mm. sartorius and gracilis using blunt-tip surgical forceps. Use self-retaining tissue retractor to further separate m. pectineus, while taking care not to damage the femoral nerve and vessels. Isolate the RFA for a length of 2 to 3 cm using blunt dissection.

PIG WEIGHT (KG)	BALLOON CATHETER SIZE	INSERTION APPROACH	DATE, AUTHOR, PMID
24-36	7 F	Left Fem. vein	2010, Filseth, 21926602
25-29	7 F	Open Chest	2012, Stenberg, 22307666
25-35	8 F	Right Fem. vein	2007, Kubitz, 17505303
27-30	14 F	Fem. vein	2013, Marshall, 23104696
31-44	7 F	RA	2002, Haney, 12151935
34.3-35.9	8 F	Fem. vein or ext. Jug. vein	2012, McCall, 22790084
38.3-43.1	8 F	Fem. vein	1998, Tayama, 9841537

Pig Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

Rotate the beveled introduction needle to follow the contour of the RFA curvature. Insert the guidewire. The insertion needle is withdrawn. Thread the introducer through the guidewire into the vessel lumen. The blunt 7-10F cannula is passed over the guidewire into the widened RFA's lumen and advanced into desired position. It is advisable to use an access port at least 2 french sizes bigger than the PV Catheter. Attach the cannula with blood sampling port to surrounding tissue, restraining its movement.

The femoral vein is located in the same adventitia, just medial to the artery. Isolate the vein using blunt dissection. Secure 7-10F access sheet in the RFV. Confirm correct catheterization by withdrawing blood from both cannulas and run the blood gas from the RFA line, then flush the catheter with heparinized saline in order to maintain catheter patency. Flush the Catheter every 5-7 min. Secure both lines with 5-0 silk to skin that is closed with sutures. It is recommended to label the arterial and venous lines.

Use the LFV to insert the balloon catheter. It is best to use an access port at least 2F sizes bigger than the balloon catheter.

Fig. 4: 5F PV Catheter with straightening introducer retracted (top) and engaged (bottom) to uncurl pigtail.

FEMORAL APPROACH (PERCUTANEOUS OR CUT-DOWN) CATHETER INSERTION

The femoral approach uses the full length of the PV Catheter upon its introduction. The Catheter is introduced through a previously established Seldinger port. Manipulation of Catheter in the abdominal and ascending aorta and also in the aortic arch requires practice to limit vascular injury and induction of clotting cascade. Also, be cautious when manipulating Catheters inside pig arteries, since they are predisposed to vasospasms. Although the major difficulty while introducing the Catheter into the LV comes when the Catheter is passing through the aortic valve, other difficulties include its misplacement into the LCA and truncus brachiocephalicus. Use of fluoroscopy guidance for this femoral access introduction is essential. Note: Make sure there is not an excessive resistance present on introduction (vasoconstriction, vessel lumen distortion). If you encounter resistance, please stop moving forward and try to carefully pull back. Use fluoroscopy to determine the cause of an obstruction before the Catheter re-introduction.

With the 5F pigtail VSL Catheters, use the pigtail straightening introducer to uncurl the pigtail tip (Fig. 4). For 7F, straighten the pigtail with a guidewire. Ideally using 5F or 7F PV Catheters, there should be a very low amount of bleeding on introduction. For this remote LV catheterization, VSL Catheters with pigtail tip (both 5F or 7F) work best as the manipulation with a straight tip Catheter usually leads to its misplacement into the above mentioned vascular structures in the aortic arch. With the Catheter in the RFA, get the feel for the degree of resistance while gently rotating the Catheter. Manipulation of the pigtail Catheter in the aortic arch is easier because it can slide down along the aortic curvature. Also, in the final destination, the pigtail can be anchored more easily in the LV apex compared to a straight tip Catheter. If necessary, a 7F closed or open pigtail Catheter can be used with an internal guidewire to manoeuvre the tip through all above mentioned structures giving the user additional capability to make a sharp left turn in the area of the ascending aorta-at the origin of the aortic arch.

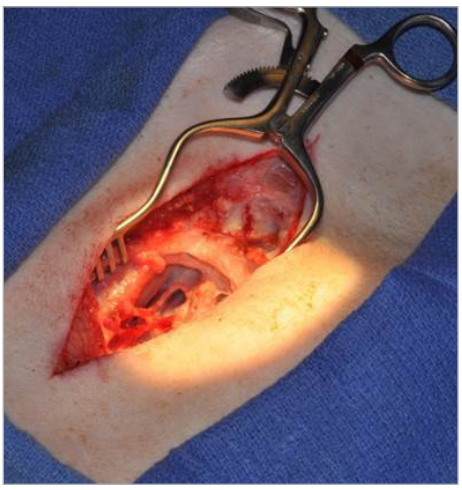

Fig. 5: Surgical site for right carotid approach

Note: If you choose to cut the end of 7F VSL closed pigtail Catheter to insert guidewire past the otherwise closed end, ensure that blood is not bleeding through the Catheter lumen. Position a hemostatic valve and/or stop cock at the end of the guidewire port.

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Pig Left Ventricle PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

RIGHT CAROTID CUT-DOWN APPROACH

Use a scalpel to make about a 9 cm skin incision perpendicular and medial to the line from the point of the jaw to the point of the shoulder. When using the el. cautery for dissection, please disconnect the PV Catheter. Use retractor to open the incision (Fig. 5). Use blunt scissors or medium hemostats to bluntly dissect an underlying glandular tissue from skin. Next, bluntly dissect the subcutaneous fascia and the fascial plane between the trachea and the sternohyoideus and the sternomastoideus mm to palpate the RCA pulse and localize the carotid adventitial sheath. Isolate using blunt dissection in order to prevent damage to the vagus nerve or its branch, the recurrent laryngeal nerve, that lies within the carotid sheath (Fig. 6). Also avoid damaging the nearby truncus sympaticus. Ligate/cauterize all arterial branches to reduce blood loss and minimize risk of dissection of the artery. Avoid major bleeding in the area. Minor bleeding can be stopped by digitally applied pressure on pre-cut gauze squares. Keep area moist with warm sterile saline or PBS.

Continue blunt dissection to expose the RCA to about 7 cm length. Dissect alongside the RCA distally towards the head. Ensure that section of the RCA is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures.

At this stage 2-0 silk sutures can be placed caudally around the RCA or internal jugular vein and ligated. To determine the anatomical location of this suture, please refer to Figure 9. Place the vessel loop to the caudal end on the RCA (close to sternum) isolated from its surrounding vascular fascia, to create tension.

Use sharp pointed scissors to make an arteriotomy (Fig. 7). A 0.038-inch guidewire is passed into RCA lumen (Fig. 8) before introducing a 7-10F blunt cannula (Fig. 9). The guidewire is then withdrawn. At this point the RCA has been retracted proximally and distally and cannulated (Fig. 10). The same process is repeated with the jugular vein.

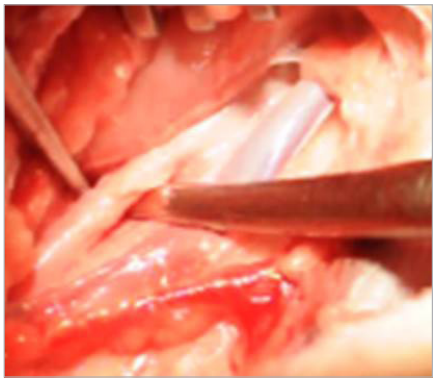

Fig. 6: Separation of the right common carotid artery

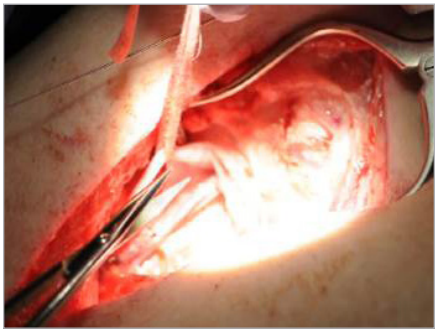

Fig. 7: Caudal control of the RCA is provided by lifting the vessel loop with exposed vascular segment in order to make an arteriotomy

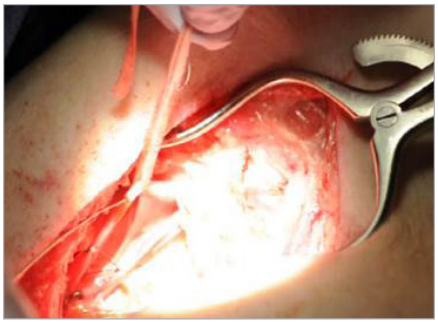

Fig. 8: Guidewire is inserted into the opening

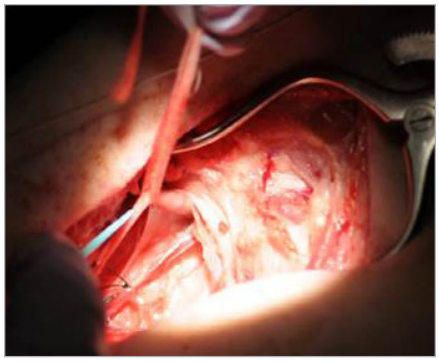

Fig. 9: Cannula is inserted over the guidewire and positioned, using the vessel loop

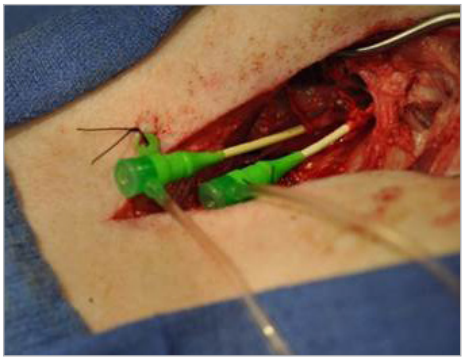

Fig. 10: Both the RCA and jugular vein are cannulated and ready for Catheter insertion

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Pig Left Ventricle PV Measurement (Closed Chest) Cont.

Measurement Procedure

CATHETER POSITION ADJUSTMENT IN LV

Before the Catheter reaches the LV, a typical arterial pressure tracing with dicrotic notch pattern, and a downward deflection on the down stroke of an arterial pressure waveform representing closure of aortic valve, can be observed (Fig. 11). On entering the LV cavity, changes in the pressure tracings occurs (Fig. 12).

Allow the Catheter to stabilize in the LV for about 15 min before data collection. Crude adjustment needs to be made at this time based on phase angle (ɵ) and admittance magnitude (Y). Both signals should measure sinusoid wave signal. In the case of an off-center position, acquired sinusoid signals might be distorted (low amplitude, frequency etc.). Reposition the Catheter to stabilize and improve the signal. For more detailed description of Catheter positioning please see "Proper PV Catheter Placement in the Left Ventricle" on page 45. Later, obtain end-systolic and end-diastolic blood conductance ($G_{b\text{-}ED}$ and $G_{b\text{-}ES}$) by turning the ventilator off for 15-20 sec. Every time after an adjustment (catheter position, ventilation, temperature etc.), re-record baseline PV. Record steady state values for at least 10 min for each animal before attempting IVC occlusion.

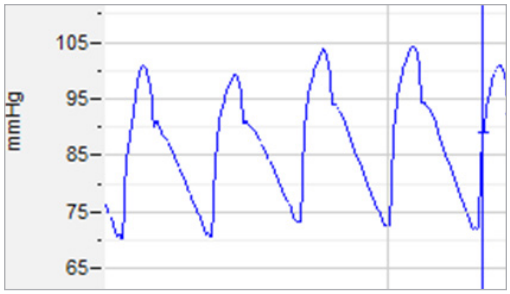

Fig. 11: Pressure trace in ascending aorta

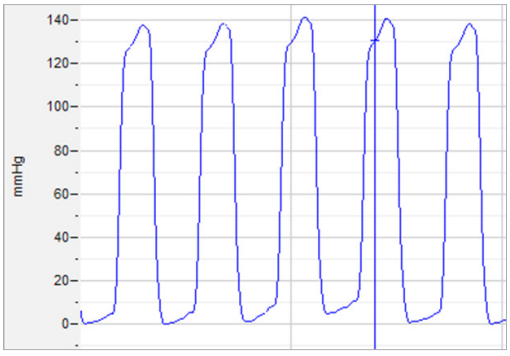

Fig. 12: Pressure trace in left ventricle

IVC OCCLUSION

Prepare the Fogarty balloon catheter and prime the inflation syringe with Iopamidol or similar contrast material. Inferior vena cava (IVC) occlusion is used to derive various load-independent indices of systolic function. Carefully insert the tip of the Catheter through the venous access port and pass the necessary length of Catheter shaft under the fluoroscopic guidance into the IVC. Localize the tip of the balloon catheter past the diaphragm (Fig. 14). Before inflating the balloon in the IVC, confirm its position by angiography.

Preload reduction: Before IVC occlusion, record pressure-volume steady state (Fig. 13). When the heart rhythm is stable, stop controlled mechanical ventilation and record baseline, uninfluenced by the lung motion artifacts. Record 5 sec of PV loop data and proceed to manometer- radio-opaque contrast dye balloon inflation to visualize the inflated balloon *in-situ* (Fig. 15). Its correct position is indicated by an immediate LV pressure and volume drop (Fig. 16). Deflate balloon and repeat these steps to obtain more than one stable occlusion, while allowing the pig to recover for at least 5 min between each IVC occlusion. If premature ventricular contractions (PVCs) occur during recording of baseline or IVC occlusion, exclude resultant PV loops. In the case of multiple incidents, all IVC occlusion recordings should be discarded and repeated.

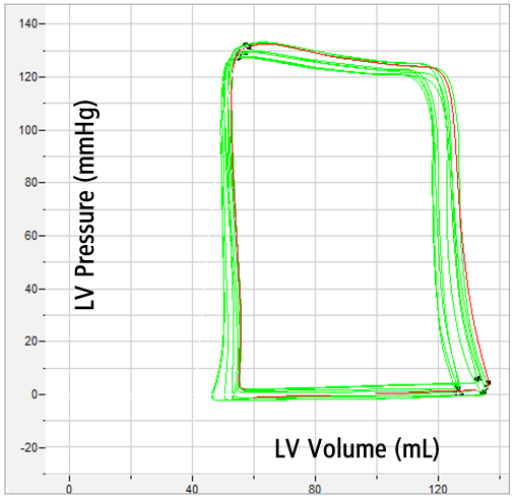

Fig. 13: Baseline (steady-state) PV loops for pig left ventricle

Pig Left Ventricle PV Measurement (Closed Chest) Cont.

Measurement Procedure Cont.

While the animal is recovering from an IVC, promptly perform offline analysis of the preload reduction using data analysis software. Start the occlusion analysis at the point at which both systolic pressure and volume decline simultaneously and end at the point at which their decline reaches a plateau. Also pay attention to heart rate. Use data where HR does not change more than 30% from baseline during the IVC occlusion.

END OF EXPERIMENT

Terminate the experiment by carefully removing the PV Catheter by gently pulling it back through the access port. Please make sure that this manoeuvre is controlled, ensuring that the Catheter shaft that houses electronics is not overly extended. Insert Catheter tip into 20 ml saline centrifuge tube. Stop digital acquisition and save the data. Clean the PV Catheter as soon as possible according to proper care guidelines to considerably prolong the Catheter's life see "Cleaning Guidelines for Catheters" on page 53.

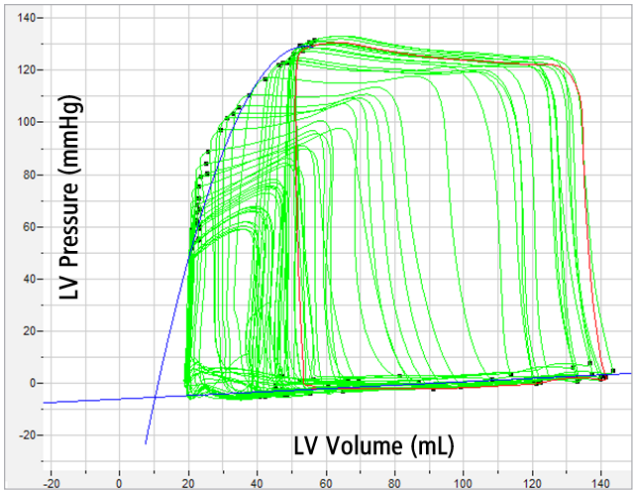

Fig. 16: PV loops during inferior vena cava (IVC) occlusion

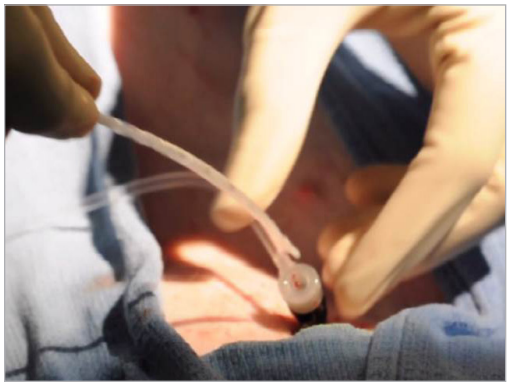

Fig. 14: Insertion of deflated Fogarty occlusion balloon catheter into the IVC through the percutaneous LFV access. The insertion port on the LFV access has to be at least 2F sizes larger to allow smooth access of the balloon catheter.

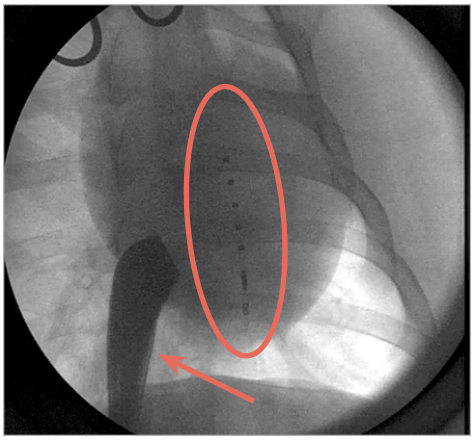

Fig. 15: Cardiac fluoroscopic image showing placement of pigtail VSL Pressure-Volume Catheter along the long axis of the left ventricle. Red arrow points to a 30 mm Fogarty occlusion balloon catheter that has been introduced through the left femoral vein (LFV) and inflated using Iopamidol to decrease preload during pressure-volume data collection.

ACKNOWLEDGMENTS

The study was approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and was in compliance with the standards in the NIH Guide for the Care and Use of Laboratory Animals.

Provided material data and measurement courtesy of Dr. Shelby Kutty, MD, FACC, and Dr. Ling Li MD, PhD RDMS, Pediatric Cardiology, University of Nebraska Medical Center, Omaha, NE 68114, skutty@unmc.edu Tel: +1 402 516 6220 Fax: +1 402 955 4356.

Transonic Scisense Inc. also appreciates the expert technical assistance of John Lof, Elizabeth Stolze, Lucas Drvol of the Joint Cardiovascular Research Laboratory of the University of Nebraska Medical Center.

Pig Simultaneous Bi-Ventricular PV Measurements (Closed Chest)

APPLICATION BASICS

Site:	Closed chest through the cut down of the Right Carotid Artery and the Right Jugular Vein
Species:	Pig (yorkshire)
Body Weight:	45 - 50 kg
Duration:	Acute

CATHETERS

Size:	7.0F
Type:	VSL Pressure Volume
Catalog #:	FTH-7018B-E248(A, B, C or D)
SYSTEM	ADV500 / ADVantage Dual Frequency Set-up:
	LV PV - FFS-097-A004 (2 kHz)
	RV PV - FFS-097-A004-C1 (25kHz)

Surgical Approach: Artery and Vein Catheterization

PERCUTANEOUS FEMORAL ARTERY AND VEIN CATHETERIZATION FOR IVC BALLOON INSERTION

Locate the right femoral artery (RFA) by using anatomical landmarks and/or pulse palpation in the femoral canal or with help of ultrasound guidance (USG). Use a large 18-gauge, 2 3/4-inch Seldinger needle or trocar and percutaneously introduce it into the arterial lumen while advancing the round tipped 0.038-inch Seldinger guidewire through the needle lumen into the RFA. Withdraw the trocar from the skin. Do not damage the Seldinger guidewire. If under USG guidance, examine position of the wire in the vessel lumen. Thread the dilator cannula, also known as sheath, over the guidewire into the RFA and advance it to desired position. A small incision might be necessary to insert the introducer past the dilator. Remove the Seldinger guidewire and introducer, leaving the blunt 7-10 F sheath with blood sampling port in the RFA. The sheath is further sutured to skin to prevent its movement (Fig 2).

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion (balloon catheter occlusion) as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Basic Set-up & Approach

The Right Carotid Artery (RCA) was cannulated using a 9F introducer for the LV 7F PV catheter.

The Right Jugular Vein (RJV) was cannulated using a 9F introducer for the RV 7F PV catheter. RV PV catheter was inserted under fluoroscopic guidance and its position was confirmed when scanning for baseline using the ADV control unit.

The Left Carotid Artery (LCA) was cannulated using a 9F introducer to place 7F pressure-only catheter into the Aortic arch to monitor the Aortic pressure.

The Left Jugular Vein (LJV) was cannulated using a 7F introducer for a Swan-Ganz catheter to measure the CVP, RAP, RVP, PAP, and PAWP (LAP). Also used to measure CO which was used to calculate SV for input into the ADV control unit prior to PV data collection.

The Right or the Left Femoral Vein (RFV or LFV) was cannulated using a 12-14 F introducer for IVC balloon insertion, Heparin dosing, CT iodine based-contrast material injection, and blood sampling.

ACKNOWLEDGMENTS

The study was approved by the Institutional Animal Care and Use Committee of the University of Buffalo, The State University of NY. and was in compliance with the standards in the NIH Guide for the Care and Use of Laboratory Animals.

Provided material data and measurement courtesy of Dr. Brian Weil, Research Assistant Professor; Department: Physiology and Biophysics; Address: 7030 Clinical Translational Research Center, UB CTRC, Suite 7030, 875 Ellicott Street, Buffalo, NY 14203 University of Buffalo, NY, bweil@buffalo.edu: Tel: +1 (716) 829-2663.

Transonic Scisense Inc. also appreciates the expert technical assistance of Elaine Granica of the Clinical Translational Research Center, UB and Cardiovascular Research Laboratory of the University of Buffalo, NY.

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Pig Bi-Ventricular PV Measurements (Closed Chest) Cont.

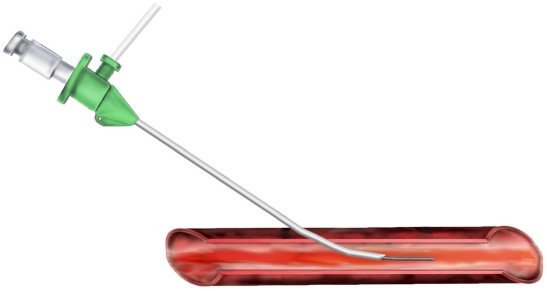

Fig. 1 Introduction of guidewire into an artery using the Seldinger technique

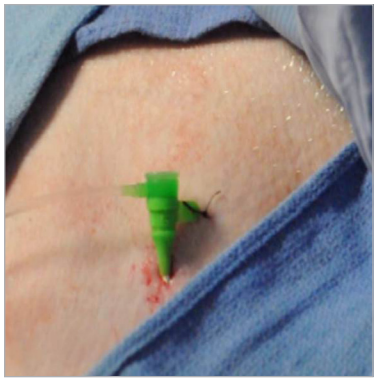

Fig. 2 Femoral vasculature access via percutaneous Seldinger technique.

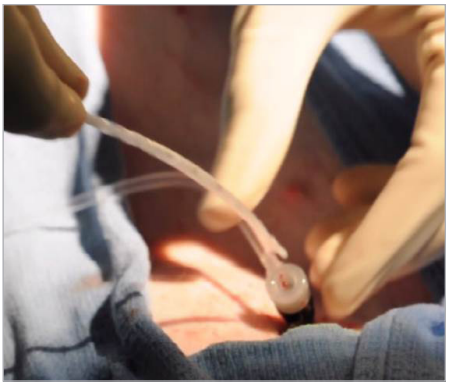

Fig. 3 Insertion of deflated Fogarty occlusion balloon catheter into the IVC through the percutaneous LFV access. The insertion port on the LFV access has to be at least 2F sizes bigger to allow smooth access of the balloon catheter.

PERCUTANEOUS FEMORAL ARTERY AND VEIN CATHETERIZATION CONT.

The femoral vein is located in the same adventitia, just medial to the artery. A 12F or 14F access sheath is secured in the femoral vein. The catheterization is confirmed by withdrawing blood and the blood gas run from the RFA line, then both catheters are flushed with heparinized saline in order to maintain patency. Catheters should be periodically flushed every 10 min. Both catheters are secured to the skin using 3-0 silk. We used the Left Femoral vein (LFV) to insert the balloon catheter. An access port, 2F larger than the indwelling sheath works best for inserting balloon catheter.

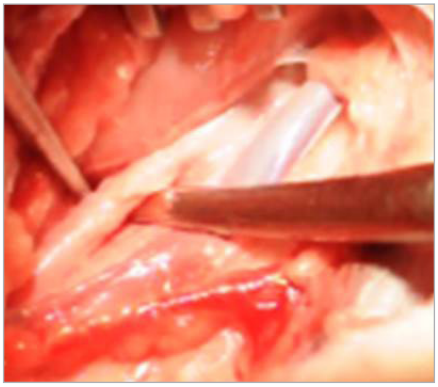

Fig. 4 Separation of the right common carotid artery

RIGHT CAROTID CUT-DOWN APPROACH

Caution: When using an electric cautery for dissection, disconnect the PV Catheter.

Use a scalpel to make an approximately 9 cm skin incision perpendicular and medial to the line from the point of the jaw to the point of the shoulder. Use a retractor to open the incision. Use blunt scissors or medium hemostats to bluntly dissect any underlying glandular tissue from skin. Next, bluntly dissect the subcutaneous fascia and the facial plane between the trachea and the sternohyoideus and the sternomastoideus mm. to palpate the RCA pulse and localize the carotid adventitial sheath. Isolate using blunt dissection in order to prevent damage to the vagus nerve or its branch, the recurrent laryngeal nerve that lies within the carotid sheath (Fig. 4).

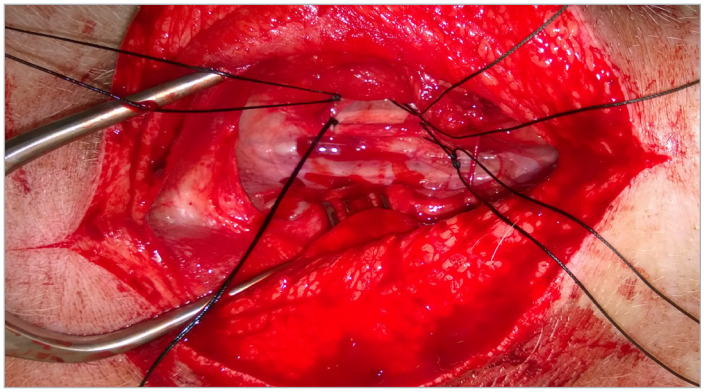

Fig. 5 The area of the ventral neck prepared for the insertion of vascular sheaths.

Also avoid damaging the nearby truncus sympaticus. Ligate/cauterize all arterial branches to reduce blood loss and minimize risk of dissection of the artery. Avoid major bleeding in the area. Minor bleeding can be stopped by digitally applied pressure on pre-cut gauze squares. Keep area moist with warm sterile saline or PBS.

Pig Bi-Ventricular PV Measurements (Closed Chest) Cont.

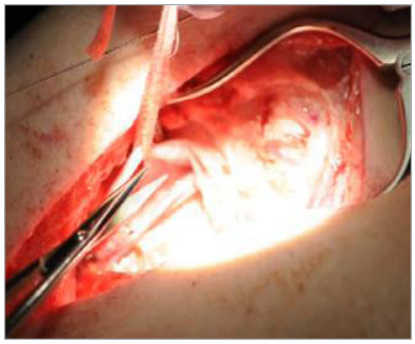

Fig. 6 Caudal control of the RCA is provided by lifting the vessel loop with exposed vascular segment in order to make an arteriotomy.

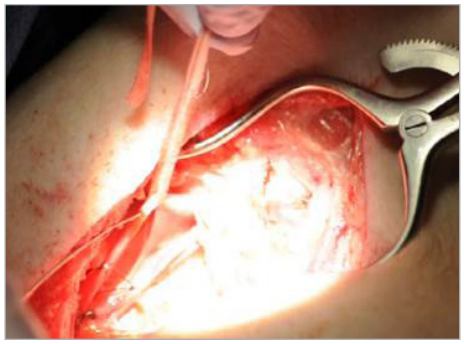

Fig. 7 Guidewire is inserted into the opening

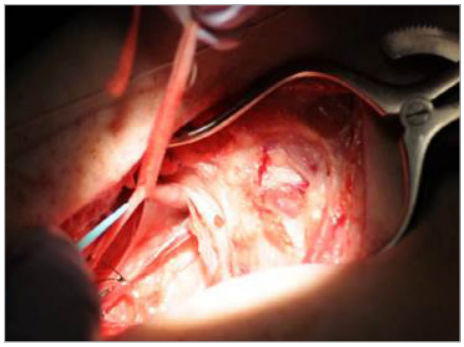

Fig. 8 Cannula is inserted over the guidewire and positioned using the vessel loop.

RIGHT CAROTID CUT-DOWN APPROACH CONT.

Continue blunt dissection to expose the RCA from vascular fascia and vagus nerve to about 7 cm. Dissect alongside the RCA distally towards the head. Ensure that section of the RCA is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures.

At this stage 2-0 silk sutures can be placed caudally around the RCA and ligated. To determine the anatomical location of this suture (Fig. 5). Place the vessel loop to the caudal end (close to sternum) to create tension on the successfully exposed RCA.

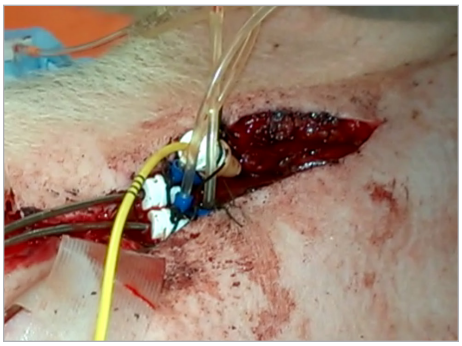

Fig. 9 Both the RCA and jugular vein are cannulated with Catheters inserted.

Use sharp pointed scissors to make the arteriotomy (Fig. 6). A 0.038-inch guide wire is passed into RCA lumen (Fig. 7) before the introduction of a 9F blunt cannula (Fig. 8). The guidewire is then withdrawn. At this point the RCA has been retracted proximally and distally and cannulated (Fig. 9).

The same process is repeated with the jugular vein. Surgical dissection of the RJV and its cannulation using the ventral neck entry provides the most direct, timely and reliable access. Ligate/cauterize all branches to reduce blood loss and minimize risk of dissection of the vein.

CONSIDERATIONS FOR CATHETER PLACEMENT

Manipulation of the catheter in the vena cava leading to cannulation of the RA and RV requires practice to limit vascular injury and prevent the induction of clotting cascades. Insertion is through an introducer 2F sizes larger than the catheter is placed in the RJV. The guidewire is partially withdrawn when the catheter is making the right turn from the superior vena cava into the RA and through the tricuspid valve. There is very small resistance as compared to the left side insertion. It is best to achieve visualization under the fluoroscopy to avoid mis-insertions into the fossa ovalis or getting caught on the papillary muscles, chordae tendinae or moderator band. The pigtail end on the catheter helps to turn and smoothly pass the catheter into the RV. Additionally, observing measurements from the pressure sensor can help confirm the location of the catheter based on the change in waveform as the catheter transitions from the RA to RV (Fig 10).

Swine arteries are predisposed to vasospasms when catheters are manipulated inside of them. To prevent vasospasms it is advisable to select anesthesia which carries less effect on the peripheral vasculature, A calcium channel blocking agent can be temporarily used

Pig Bi-Ventricular PV Measurements (Closed Chest) Cont.

CONSIDERATIONS FOR CATHETER PLACEMENT CONT.

to help cannulate. Additionally, make sure that there is no excessive resistance during catheter introduction due to vessel lumen distortion. If you encounter resistance, stop moving forward and try to carefully pull back. Use fluoroscopy to determine the cause of the obstruction before the catheter introduction.

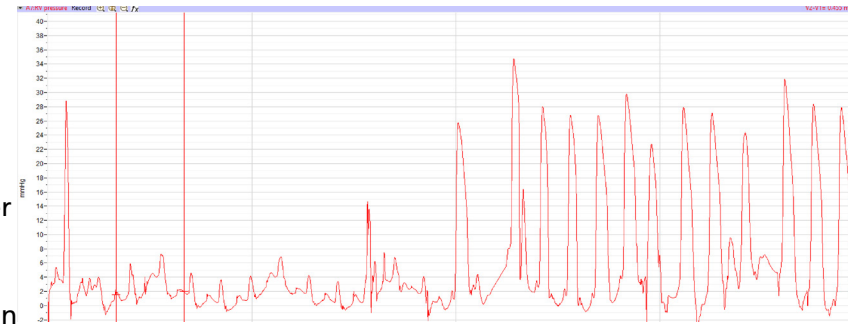

Fig. 10 Showing the RAP to RVP transfer with slight presence of breathing artifact.

For 7F catheter insertion into the LV, straighten the pigtail with a guidewire when passing through the hemostatic valve and later the vascular sheath. There should be a small amount of bleeding on introduction. With the catheter in the RCA, envision the anatomy of the Aortic arch to be able to insert the catheter through this imaginary path into the LV.

If the degree of resistance on insertion is still very high, try to gently rotate the catheter in the RCA. If the rotation is not possible, do not proceed and withdraw the PV catheter. As the catheter houses electronics, every kink and bend carries the risk of detachment of the blood pressure sensor in the catheter's shaft. It is advisable to use fluoroscopy during the early insertion to avoid any excessive force on cannulation. It may be necessary to withdraw the catheter and adjust the animal on the surgical table to find the best path to cannulate.

HEMODYNAMIC PARAMETERS (SWINE)	LV VALUES		RV VALUES	
	MEAN	SD	MEAN	SD
HR (bpm)	127	1.5	127	1.5
ESP (mmHg)	87.6	2.0	33.5	3.6
EDP (mmHg)	3.9	0.8	6.3	1.8
dPdt max (mmHg/s)	1966	59	447	11
dPdt min (mmHg/s)	-1916	34	-531	8
ESV (ml)	26.8	3.5	31.8	1.3
EDV (ml)	67.3	2.3	68.6	2.7
SV (ml)	40.5	1.0	36.8	2.7
CO (ml/min)	5149	84	4674	391
EF (%)	60.2	1.7	53.9	2.4
SW (Joules)	0.23	0.01	0.08	0.01

The main difficulty while introducing the PV catheter into the LV comes when the catheter is passing through the Aortic valve. In catheters with closed lumen, the guidewire can be pulled back and the pigtail will create larger pushing area enabling the catheter to overcome the Aortic valve resistance. LV PV catheter should be manipulated under fluoroscopic guidance to avoid its damage and any mis-insertions.

In the case of open lumen catheters, when the pigtail end is cut, the guidewire can be introduced first and the catheter will follow the guidewire into the LV. Using the internal guidewire can also help to manoeuvre the tip through all the above mentioned structures giving the user additional capability to make a sharp turn in the area of the ascending aorta at the origin of the aortic arch. In this introduction, fluoroscopy guidance helps to properly position of the pigtail end into its final destination, and the pigtail can be anchored more easily in the LV apex. When the pigtail tip of the 7F catheter is cut, please make sure that hemostatic valve (FA-70HV1) is placed before the catheter enters the vasculature as blood under pressure might spurt through the open lumen. For how to place a hemostatic valve please see our 7F Catheter Guide.

SWINE (40-60 KG)	LV RANGES OF VALUES	RV RANGES OF VALUES
Systolic Pressure	70-100 mmHg	20-35 mmHg
Diastolic Pressure	1-6 mmHg	1-6 mmHg
Phase Amplitude	1-5 °(degrees)	2-7 °(degrees)
Mean Phase	1.5 °(degrees)	close to 1.5 °(degrees)
Magnitude Amplitude	over 12 up to 30 mS	over 9 up to 20mS
Mean Magnitude	≥2.5 mS	over 2 mS
Blood resistivity	1.5 Ohm*m	1.5 Ohm*m
Heart Rate	70-150/min (bpm)	70-150/min (bpm)

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THE MEASURE OF BETTER RESULTS.

Pig Bi-Ventricular PV Measurements (Closed Chest) Cont.

CATHETER POSITION ADJUSTMENT IN LV

Before the catheter reaches the LV, you can observe the typical arterial pressure wave tracing with the dicrotic notch pattern; the downward deflection on the down stroke of an arterial pressure wave represents the closing of the aortic valve (Fig. 11). On entering the LV cavity, a change occurs in the pressure trace to show a typical waveform from the left ventricle (Fig. 12).

Allow the catheter to stabilize in the LV for about 15 min before collecting data. Crude adjustment needs to be made at this time based on phase angle (ɵ) and admittance magnitude (Y). Both signals should measure sinusoid wave signals. In case of off-center position, acquired sinusoid signals might be distorted (low amplitude, frequency etc.). Reposition the catheter to stabilize and improve the signal. For more detailed description of catheter positioning please see CDS 2.03 PV Catheter Positioning Guide. Later, obtain end-systolic and end-diastolic blood conductance (Gbed and Gbes) by turning the ventilator off for 15-20 sec. Always re-record baseline PV data with every adjustment (catheter position, ventilation, temperature etc.). Record steady state values for at least 10 min for each animal before attempting IVC occlusion.

IVC OCCLUSION

Inferior vena cava (IVC) occlusion is used to derive various load-independent indices of systolic function. Prepare the Fogarty balloon catheter and prime the inflation syringe with Iopamidol or similar contrast material. Carefully insert the tip of the Catheter through the venous access port and pass enough Catheter shaft into the IVC under the fluoroscopic guidance to localize the tip of the balloon catheter past the diaphragm. Before inflating the balloon in the IVC, confirm its position by angiography.

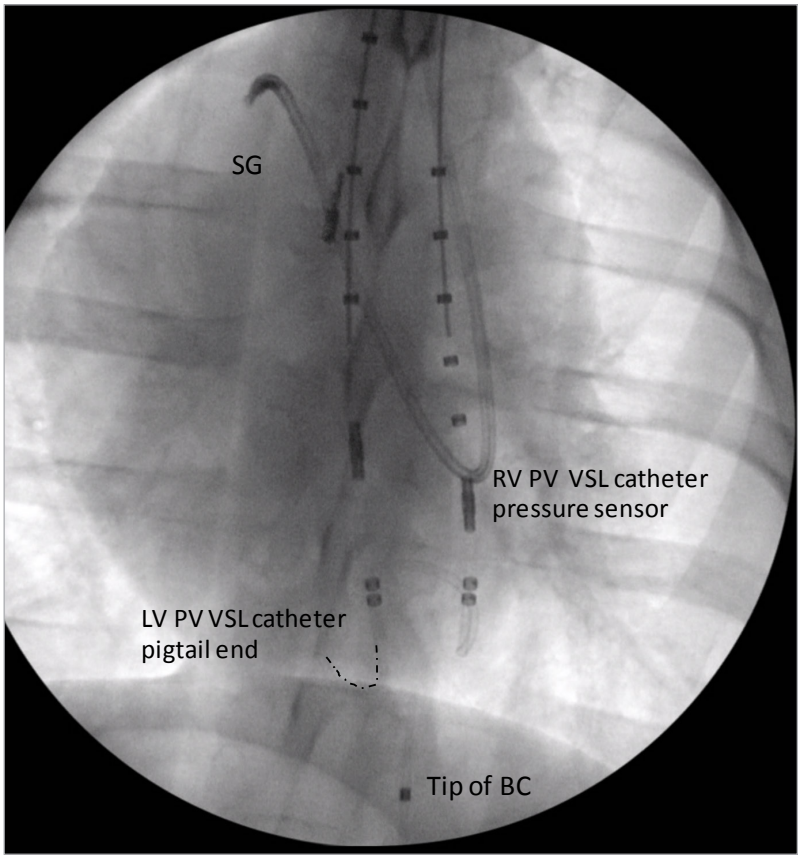


Fig. 13 X-ray of heart showing the positions of the RV catheter, LV Catheter, Swan-Ganz (SG) Catheter and Balloon Catheter (BC). Imaged using AP view.

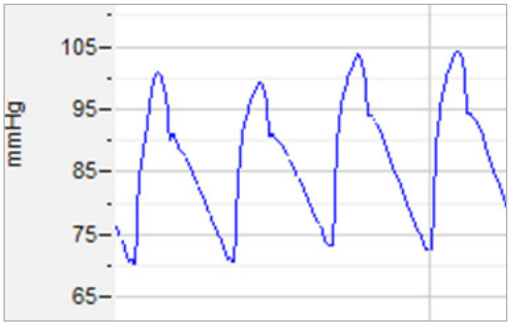


Fig. 11 Pressure trace in ascending aorta

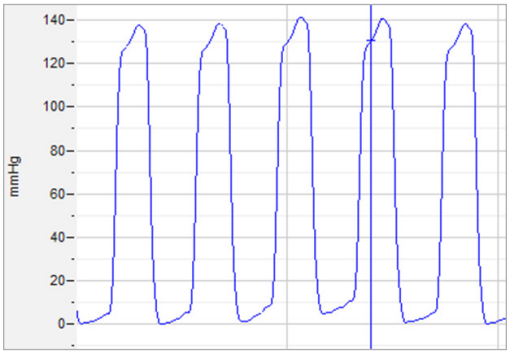


Fig. 12 Pressure trace in left ventricle

Pig Bi-Ventricular PV Measurements (Closed Chest) Cont.

IVC OCCLUSION CONT.

Preload reduction: Before IVC occlusion, record the pressure-volume steady state (Fig. 16). When the heart rhythm is stable, stop controlled mechanical ventilation and record baseline, uninfluenced by the lung motion artifacts. Record 5-10 sec of PV loop data and proceed to manometer- radio-opaque contrast dye balloon inflation to visualize the inflated balloon in-situ. Its correct position is indicated by an immediate LV pressure and volume drop (Fig. 15). Deflate the balloon and repeat these steps to obtain more than one stable occlusion, while allowing the swine to recover for at least 5 min between each IVC occlusion. If premature ventricular contractions (PVCs) occur during recording of baseline or IVC occlusion, exclude the resultant PV loops. In case of multiple incidents, all IVC occlusion recordings should be discarded and repeated.

Promptly, while the animal is recovering from an IVC, perform offline analysis of the preload reduction using data analysis software. Start the occlusion analysis at the point at which both systolic pressure and volume decline simultaneously and end at the point at which their decline reaches a plateau. Also pay attention to heart rate. Use data where HR does not change more than 30% from baseline during the IVC occlusion.

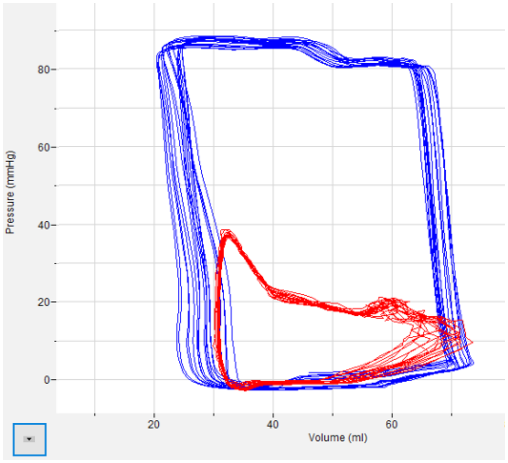


Fig. 14 Baseline PV loops for the RV (red) and the LV (blue)

END OF EXPERIMENT

Terminate the experiment and carefully remove the PV Catheters by gently pulling them back through their respective access ports. Please make sure that this maneuver is controlled, ensuring to not overly extend the Catheter shaft as it houses electronics. Immediately after removal, insert the Catheter tip into a container with warm saline. Stop digital acquisition and save the data. Follow the directions in CSD 2.06 Catheter Cleaning & Disinfecting Guide for proper cleaning before storage. Proper care of the Catheter considerably prolongs its life.

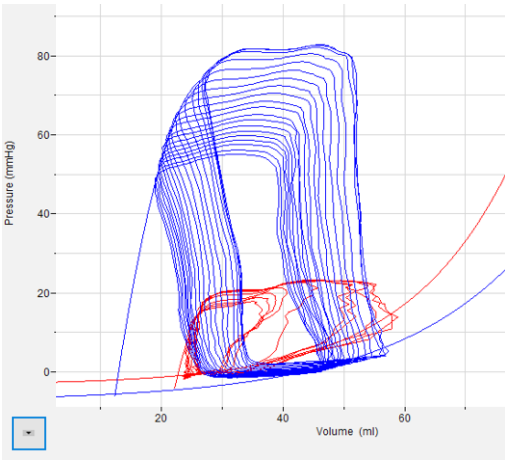


Fig. 15 IVC occlusions PV loops for the RV (red) and the LV (blue)

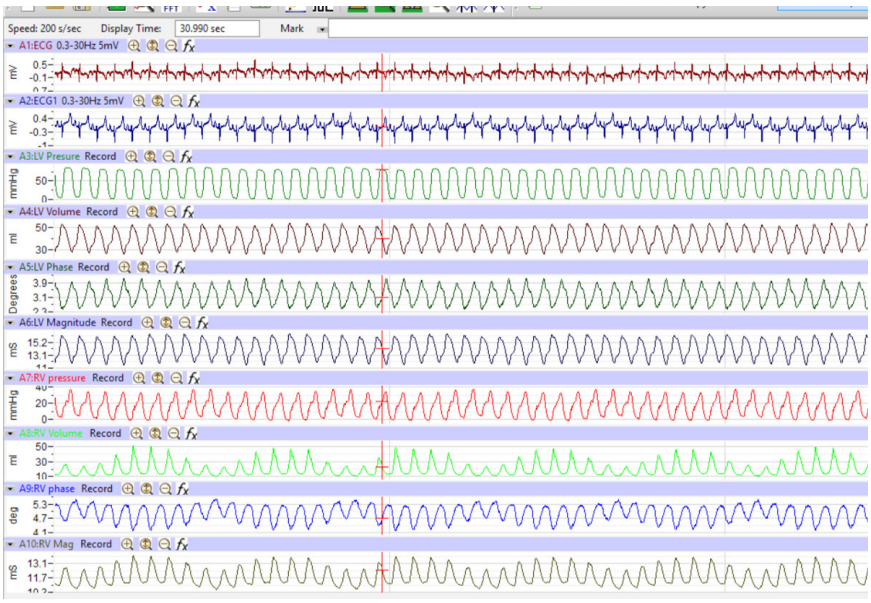


Fig. 16 Baseline data. First 2 channels are ECG, then LVP, LVV, LV Phase and LV magnitude sixth. Channel seven is RVP then RVV, RV Phase and finally RV magnitude.

Miss-inserting the Catheter through the Aortic Valve

Catheterization of the left ventricle (LV) in large animal models, e.g. swine and cattle, requires proper guidance. Correct positioning through the aortic valve is crucial for quality pressure and pressure-volume signals and the ability to confidently measure and monitor cardiac pressure and PV function.

It is important to select an appropriate Catheter for the procedure. Transonic Scisense offers large animal Pressure and PV Catheters (5.0 and 7.0 F) that are able to be inserted and positioned in the LV through the right carotid artery using Seldinger insertion or by an alternative approach. Some invasive cardiologists/ principal investigators prefer to use 7.0 F Catheter with a 0.35" guide-wire inserted into the lumen of the Catheter, others are more comfortable inserting a 5.0 F Catheter without a guide-wire. Regardless, Transonic Scisense suggests using fluoroscopy, transthoracic (TTE) echocardiography, or transesophageal (TEE) echocardiography guidance for both Catheter insertion and its positioning within vasculature to avoid unnecessary damage of vascular and cardiac structures.

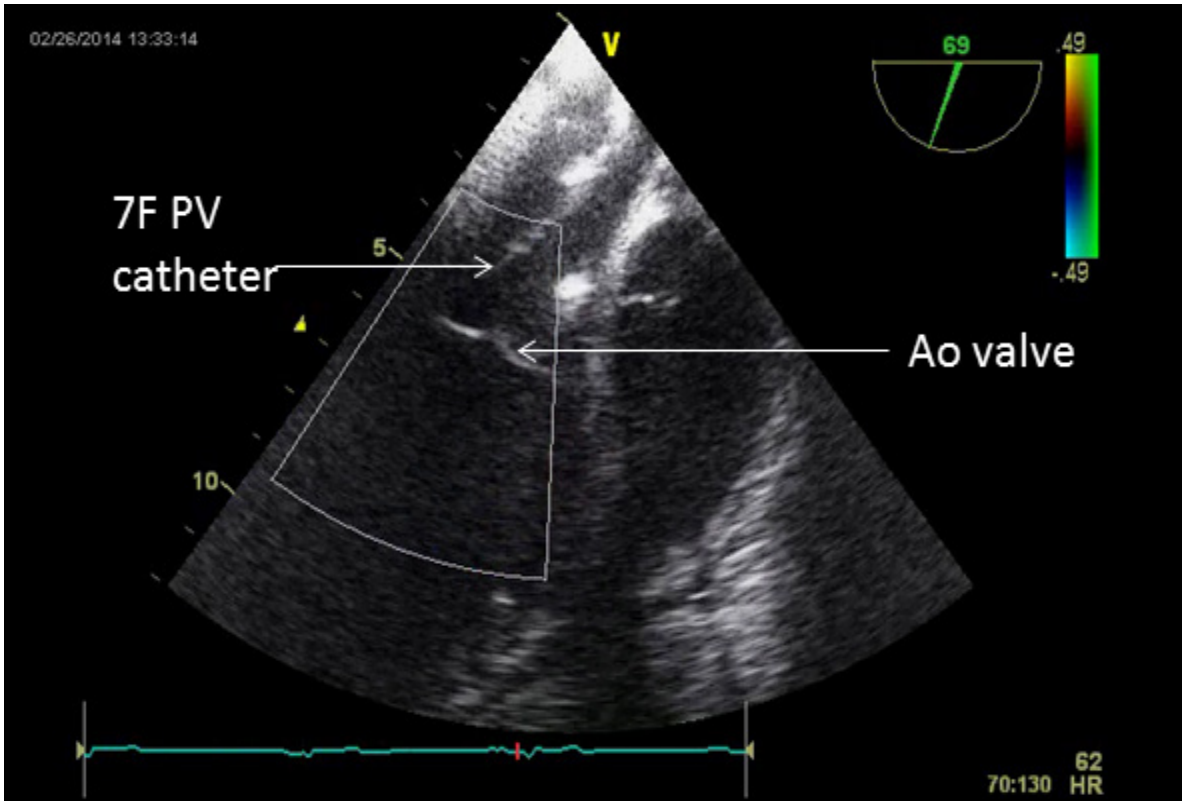


Fig. 1: This TEE image depicts a 7.0F Pressure-Volume Catheter that has been partially withdrawn into aorta after piercing through the aortic valve. Originally, the Catheter was inserted through the right carotid artery into swine's LV with a fully extended 0.35" guidewire but without direct fluoroscopy guidance.

Miss-inserting the Catheter through the Aortic Valve Cont.

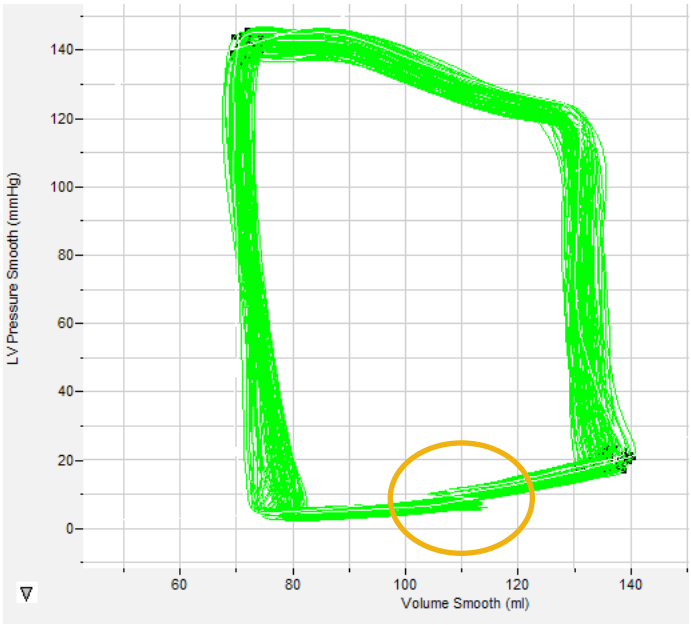


Fig. 2: Representative PV loops originated from the situation where the PV Catheter entered and pierced the aortic valve cusp. Presence of an artefact, encircled, can be localized at filling phase of LV cardiac cycle. Additionally, increased value of EDP can be observed.

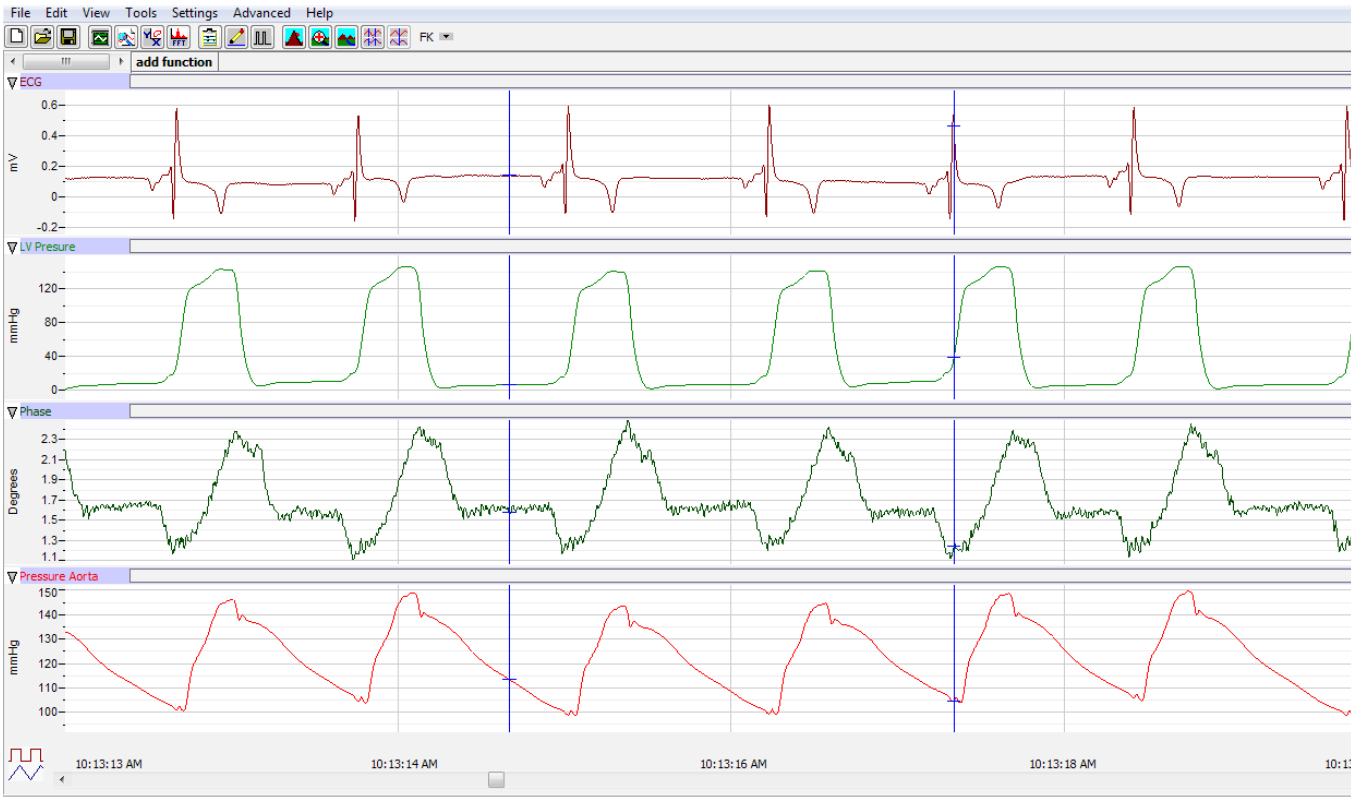


Fig. 3: Left ventricle pressure (LVP) and aortic pressure (AoP) waveforms along with the ECG trace are not showing any abnormalities except higher EDP on the LVP trace (second blue cursor). Atypical phase waveform, representing the abnormal PV Catheter location, as observed by its non-sinusoidal trace indicates PV Catheter that is moving trapped within the aortic cusp.

Miss-inserting the Catheter through the Aortic Valve Cont.

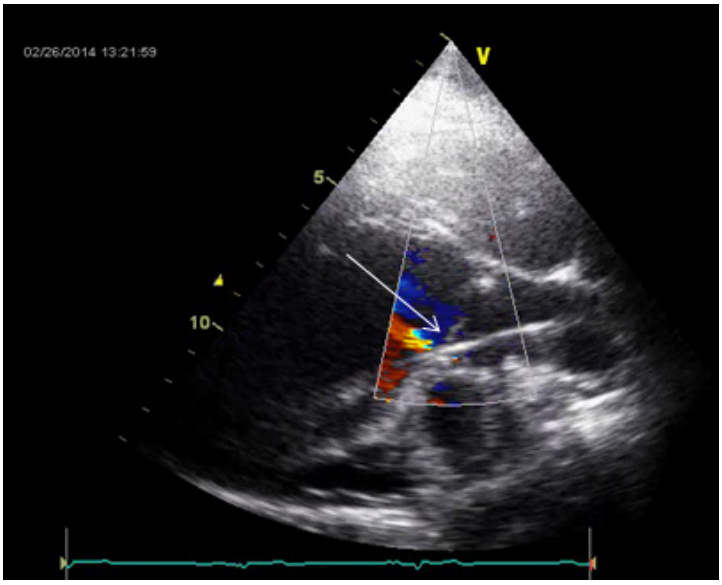


Fig. 4: TEE image of 7.0 F PV Catheter in the aortic root. Arrow points to where the Catheter entered the cusp.

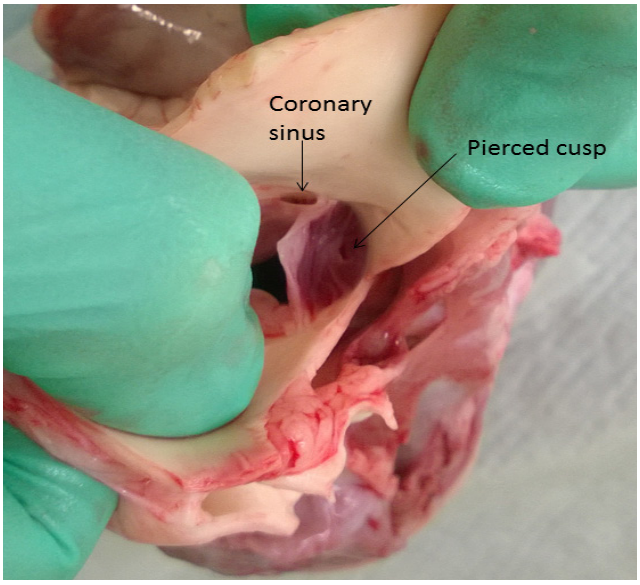
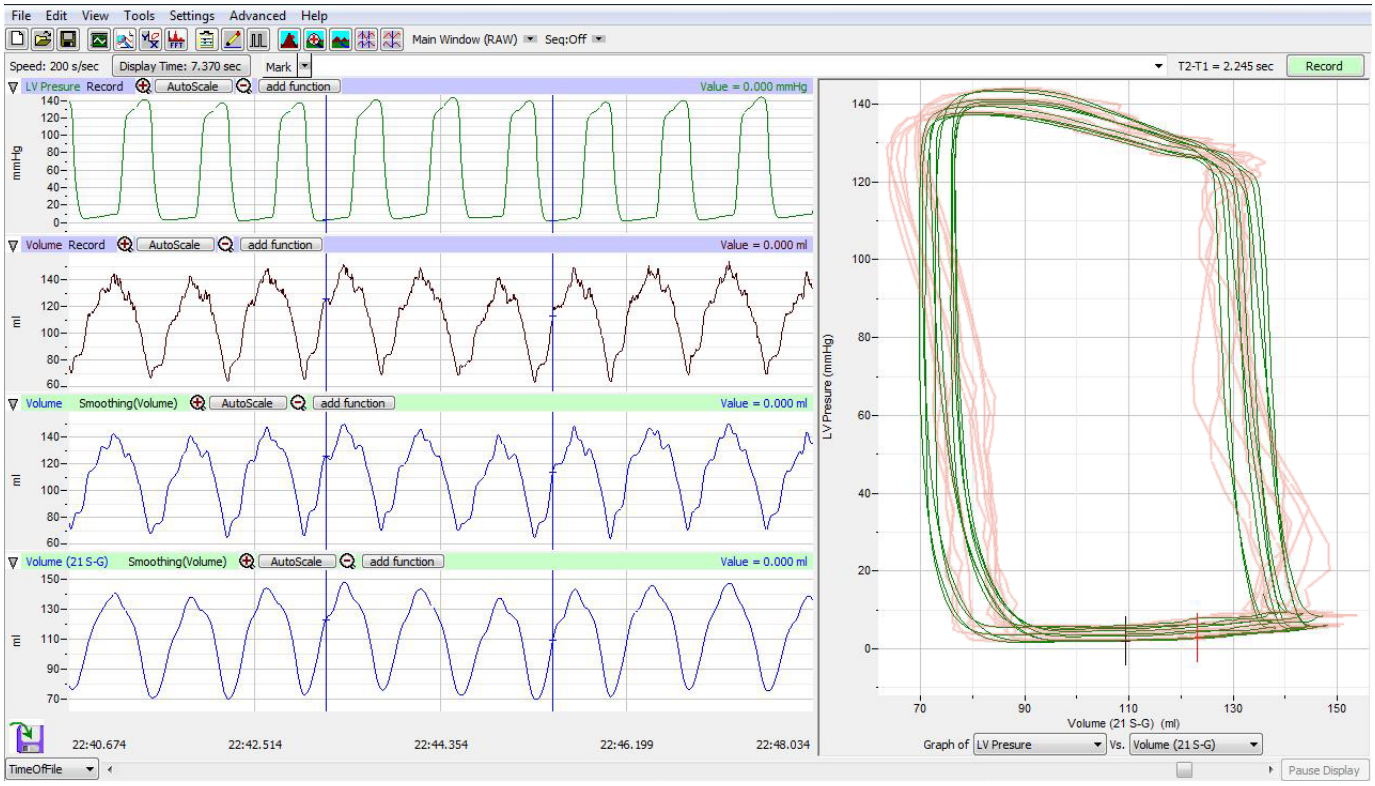


Fig. 5: Post-mortem localization of the pierced cusp of aortic valve.

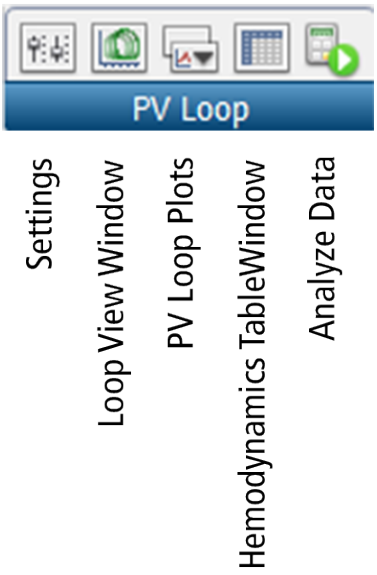
Data Analysis & Post-Processing



Analyzing PV Loop Data in LabChart

The following document outlines the essential and suggested steps for analyzing PV loop data in LabChart software when used in conjunction with the ADV500 PV System. Every experiment is different and may require different settings or the use of different software features. Please contact ADInstruments directly for additional information about all the available features or any software functionality issues

For the purpose of this exercise, a sample file recorded from a healthy mouse using the ADV500 PV system will be used. Only raw data was recorded at the time of the experiment, therefore, this example does not show hemodynamic measurements as calculated channels over time. Remember, that these channels can be created during analysis if required to show trends over the course of the experiment.



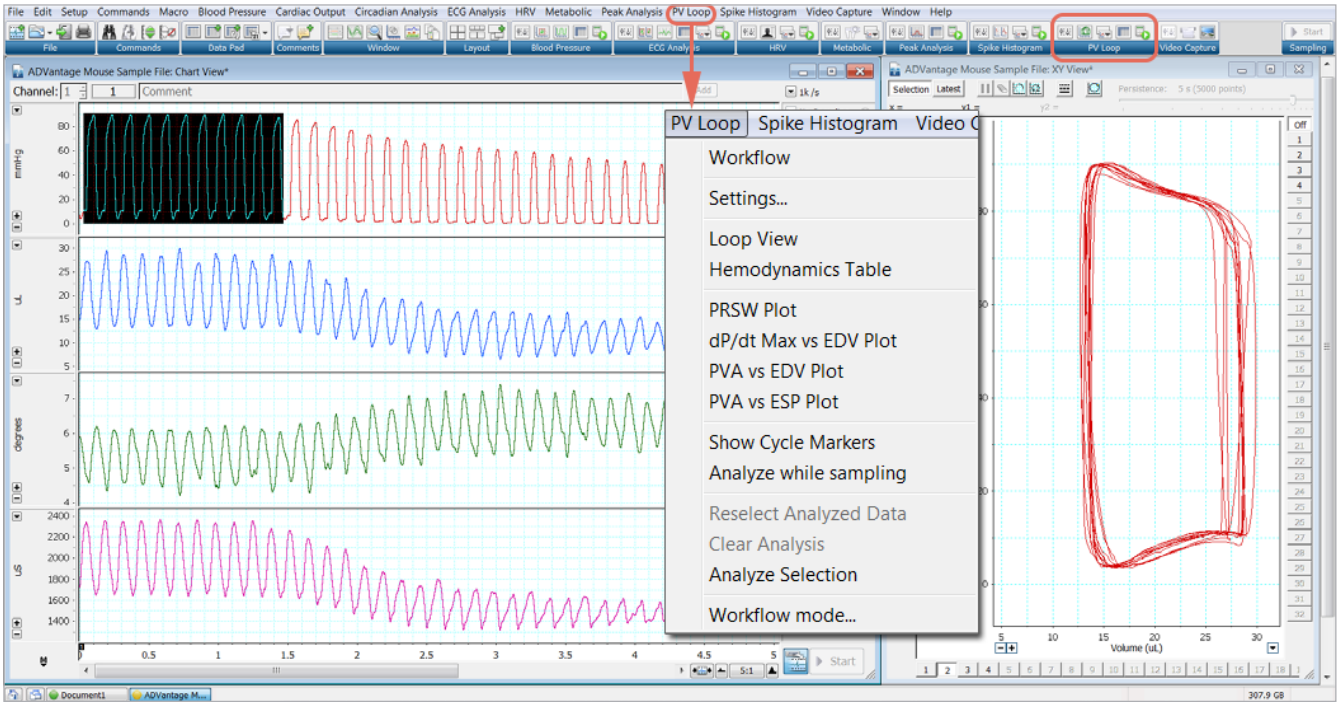
Close up view of PV Loop Hotkey menu

GETTING STARTED

Open up LabChart Software. Upon start-up a dialog box will appear and run through a diagnostic to confirm connection to PowerLab hardware. Connection to the PowerLab is not essential for data analysis: therefore, if no hardware is connected simply click OK to proceed.

The Welcome Centre will appear. Select the data file of interest from the “Recent Files” column, or click the OPEN icon to search for the file of interest.

After selecting the file it will open in the LabChart Main Window.



Main window showing mouse left ventricle PV loop data. PV Loop menu is circled and the expanded view shown. PV Loop hotkeys are circled and the expanded view is shown above.

Analyzing PV Loop Data in LabChart Cont.

PV Loop Menu & Functions

This menu and its sub-menus include all setting, preferences, or functions that one will need to address in order to ensure correct PV data calculation.

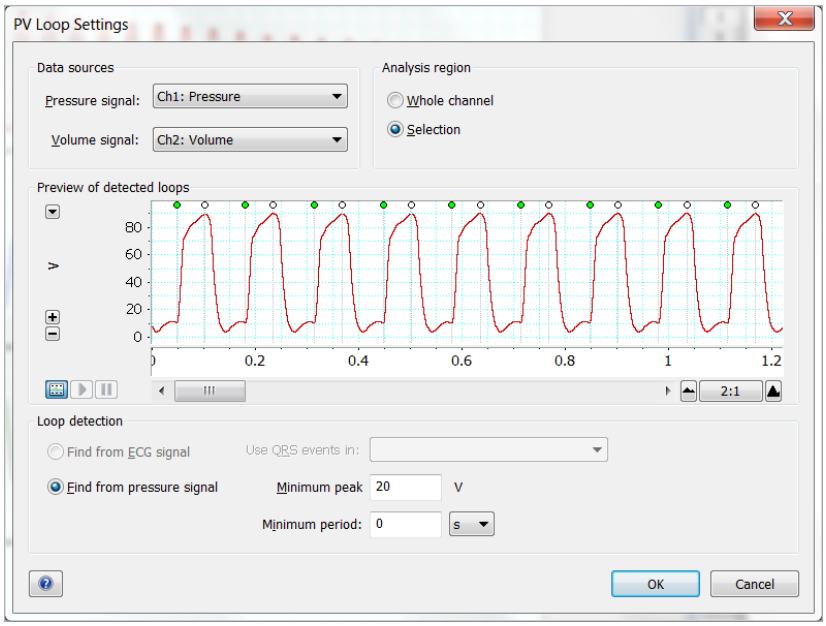
WORKFLOW

Click to activate the Workflow function. Workflow will appear at the left hand side of the screen. This is not required for analysis of admittance data as True-Volume information is already available. However, if traditional volume calibration info needs to be addressed (i.e. Compute parallel volume (Vp) from a saline bolus infusion data set), Workflow will provide assistance with these steps. Consult the ADI LabChart User Manual for specific details on Workflow.

SETTINGS

Open from the menu or hot-key to access the PV Loop Settings Dialog box. Ensure the following are correctly selected before moving forward with analysis:

1. Pressure Signal: select the appropriate Pressure Channel
2. Volume Signal: select the appropriate Volume Channel
3. Analysis Region: by selecting "Whole Channel" all registered PV cycles will be automatically included in the hemodynamic table. Typically this is not preferred, as families of specific loops will want to be studied. By choosing "Selection", you will be able to highlight areas of interest in the Main Window which will then populate in the hemodynamic table.



PV Loop Settings Dialog Box

4. Preview of Detected Loops: this section allows the researcher to confirm that each heart beat is being registered correctly as a cycle. Green Dot markers denote End Diastole, while Yellow Dot markers denote End Systole.
5. Loop Detection: this section allows the user to define how cycles will be registered by the software. The default setting is "Find from pressure signal" and is the best choice.
6. Minimum Peak and Minimum Period: These two settings allow the researcher to adjust the threshold by which the software registers a cardiac cycle.

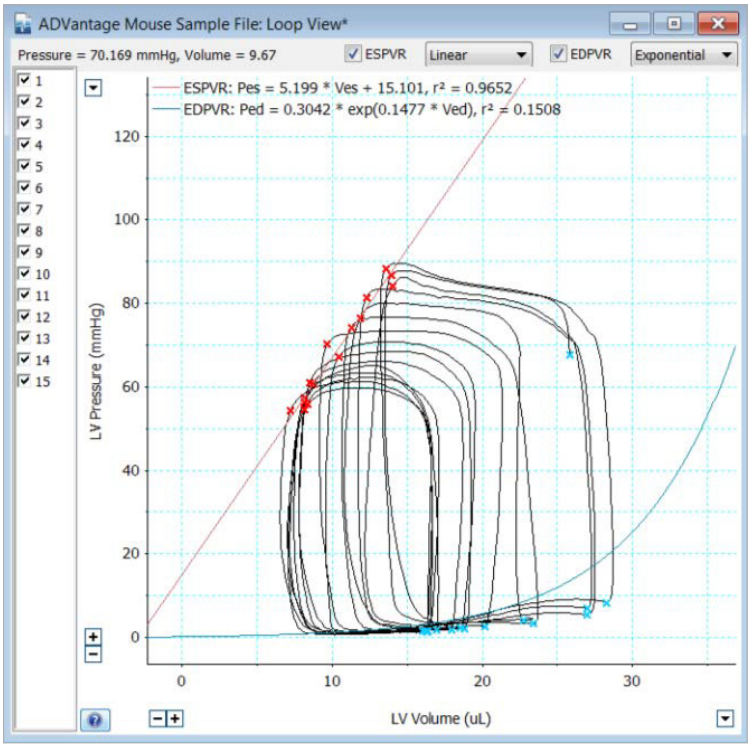
SHOW CYCLE MARKERS

By selecting this option, cycle markers (Green and Yellow tags marking end diastole and end systole, respectively) will appear in the Main Window view. This will help to confirm that the software has correctly registered each cardiac cycle for analysis.

Analyzing PV Loop Data in LabChart Cont.

LOOP VIEW

Clicking this option in the menu or the hot-key will open up the Loop View Window. This XY-Plot will contain the data selected from the Main Window (or entire data set if "Whole Channel" is selected in the Settings menu). Loop View displays the detected pressure volume loops as an XY line graph of pressure against volume. The End Systolic Pressure Volume Relationship (ESPVR) and the End Diastolic Pressure Volume Relationship (EDPVR) regression information are also displayed.



Loop View showing pressure and volume in an XY plot. ED and ES points are shown. ESPVR and EDPVR lines are shown. Included loops are listed on the left.

1. Instantaneous Readouts: To display readouts of instantaneous pressure and volume, move the tracking cursor over the end diastolic (ED) and end systolic (ES) points.
2. Loop Selection: You can highlight a particular loop in the display area, and select the loops that you would like included in the analysis.
   a. To identify a specific loop, click on the ES or ED point, or click on the loop number on the left hand side of the view. This highlights the loop in blue. The corresponding row of calculated parameters in the Hemodynamics Table is also highlighted.
   b. Only loops with a check in the box next to their loop number are included in the analysis. By default all loops are included. To exclude a loop from the analysis, deselect it by clicking on the check mark in the corresponding box. Deselecting a loop removes it from all views and calculations
3. Regression Information: The ESPVR and EDPVR best fit regression can be drawn on the graph and the regression information displayed at the top of the plot. To display this information check the boxes to the left of the ESPVR and/or EDPVR labels above the plot. For ESPVR, a linear or quadratic curve fit can be calculated and for EDPVR, a linear or exponential curve fit can be calculated (NOTE: Linear option is only there to force a calculation in the event of negative EDP values).

HEMODYNAMICS TABLE

Clicking this option in the menu or hot-key will open the Hemodynamics Table Window. Beat-by-beat information will be displayed in a tab-delimited format for all selected loops.

NOTE: In "Analyze While Sampling" mode, the contents of the parameters table are updated upon detection of each loop during data acquisition. Use the Scroll/Review button to toggle between automatic scrolling of newly added rows and reviewing of previously added rows. This feature can be used when data is being recorded at the bench top, but does not apply to pre-recorded data.

Analyzing PV Loop Data in LabChart Cont.

When analyzing pre-recorded data the table's contents are updated to show the data for the current Selection or for Whole Channel recorded data.

ADVantage Mouse Sample File: Hemodynamics Table*

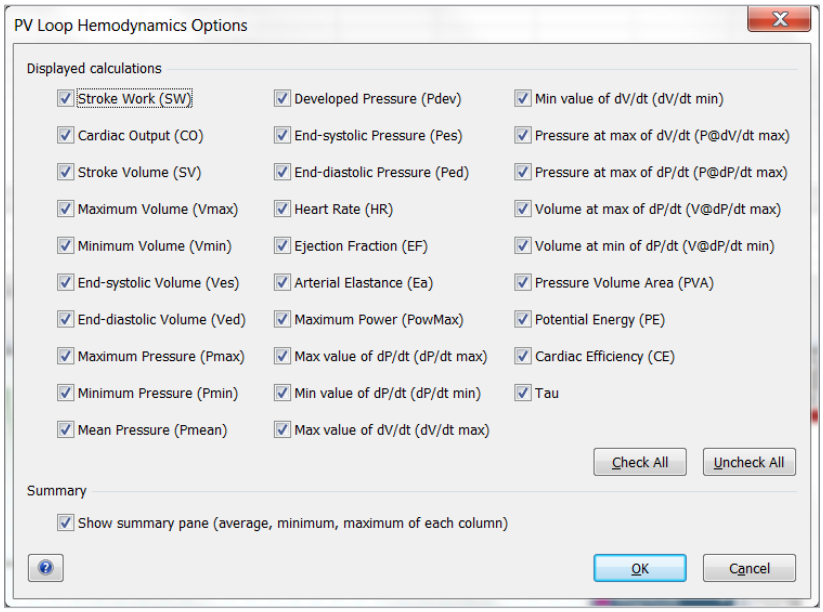
	SW (mmHg*u	CO (uL/min)	SV (uL)	Vmax (uL)	Vmin (uL)	Ves (uL)	Ved (uL)	Pmax (mmHg)	Pmin (mmHg)	Pmean (mmHg	Pes (mmHg)	Ped (mmHg)	HR (bpm)	EF (%)	Tau (ms)
1	1065	6416	14.22	27.22	13.00	13.98	27.13	89.60	3.824	44.73	86.67	10.70	451.1	52.64	8.209
2	1139	7113	15.89	29.42	13.53	14.12	26.81	90.05	3.851	44.53	87.83	10.46	447.8	55.13	8.457
3	1112	6912	15.32	28.57	13.25	13.50	28.49	89.93	3.824	44.86	88.59	10.50	451.1	54.04	8.080
4	1121	6868	15.22	28.75	13.53	14.24	28.40	89.96	3.675	44.74	88.28	10.39	451.1	53.32	8.079
5	1119	6755	15.09	28.67	13.58	13.81	28.08	90.19	3.635	44.62	89.04	10.15	447.8	53.44	8.128
6	1188	7307	16.20	29.88	13.69	14.62	28.89	90.09	3.891	45.03	88.26	10.64	451.1	54.23	8.127
7	1031	7076	15.69	28.82	13.13	13.55	26.33	90.09	3.851	44.88	88.34	10.54	451.1	58.65	8.494
8	1192	7297	16.30	28.94	12.64	13.25	28.90	90.36	3.972	44.63	86.86	10.39	447.8	57.94	8.464
9	1186	7047	15.74	28.57	12.83	13.60	28.31	90.01	3.851	43.86	88.59	10.27	447.8	55.43	8.689
10	995.0	6671	14.90	28.12	13.22	13.60	27.29	89.69	3.648	42.86	88.25	9.411	447.8	57.13	9.109

	SW (mmHg*u	CO (uL/min)	SV (uL)	Vmax (uL)	Vmin (uL)	Ves (uL)	Ved (uL)	Pmax (mmHg)	Pmin (mmHg)	Pmean (mmHg	Pes (mmHg)	Ped (mmHg)	HR (bpm)	EF (%)	Tau (ms)
Avg	1115	6946	15.46	28.70	13.24	13.83	27.86	90.00	3.802	44.47	88.07	10.35	449.4	55.20	8.384
Min	995.0	6416	14.22	27.22	12.64	13.25	26.33	89.60	3.635	42.86	86.67	9.411	447.8	52.64	8.079
Max	1192	7307	16.30	29.88	13.69	14.62	28.90	90.36	3.972	45.03	89.04	10.70	451.1	58.65	9.109
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Export... Options... Add to Data Pad

Hemodynamics Table window displays individual loop data and summary values from all selected loops.

1. Loop Selection: Clicking on a row highlights that row of data and also highlights the related loop in Loop View. To select which loops are included/ excluded in your analysis, use the Loop View.
2. Column Format: Double clicking on the column heading opens the Column Format dialog. In this dialog you can select the number of significant figures the parameters are displayed with and apply it to all the columns.
3. Options and Summary Information: The Hemodynamics Table can display:
   a. Columns for any of the calculated parameters. To select the parameters to be displayed, open the Hemodynamics Options dialog by clicking Options at the bottom of the Hemodynamics Table.

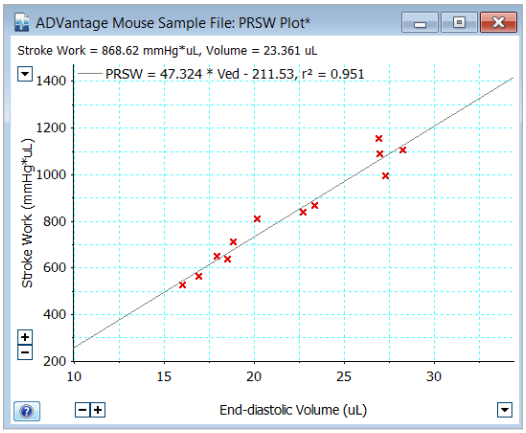


Hemodynamics Table Options dialog box for selecting which parameters are displayed.

   b. Summary rows at the bottom of the table show the average, minimum and maximum values, and the number of values, for each column. To turn on summary rows, click Options and select "Show summary pane" in the Hemodynamics Options dialog.

Analyzing PV Loop Data in LabChart Cont.

4. Exporting Data: There are two methods for exporting the contents of the Hemodynamics Table:
   a. Click Export, then select either tab-delimited text file (.txt) or comma-delimited text file (.csv) and click Save. The output file can be imported into spreadsheet programs.
   b. In the hemodynamics table, select the data of interest by holding down the left mouse button and dragging the cursor. Once the data is selected (highlighted in blue), press CTRL-C on the keyboard. Data will be transferred to the Clipboard. Open up an appropriate spreadsheet program, select the cell where you wish to import the first data point and press CTRL-V on the keyboard. NOTE: individual loop data and Summary data (AVG, MIN, MAX, COUNT), must be copied separately.

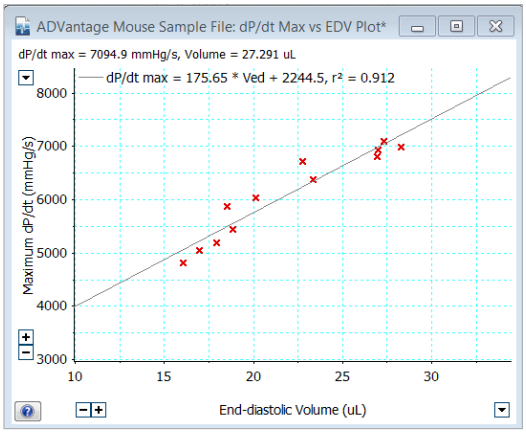


PRSW: Stroke Work vs End Diastolic Volume

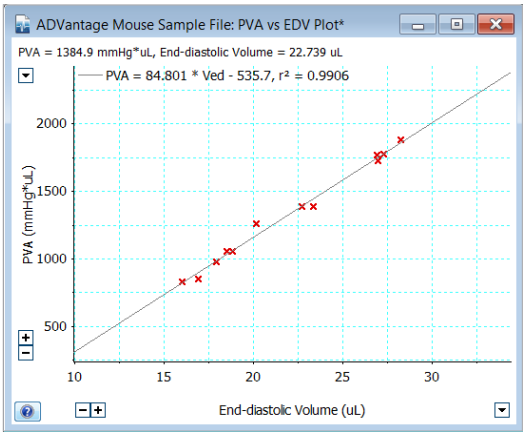
PV LOOP PLOTS

The next section of the menu lists the four available load-independent PV loop plots:

1. PRSW: Preload recruitable stroke work (PRSW) is determined by the linear regression of stroke work with the end diastolic volume. The slope of the PRSW relationship is a highly linear index of myocardial contractility that is insensitive to preload and afterload. During heart failure, myocardial contractility is reduced which decreases the slope of the PRSW relationship. Recent studies also indicate that the volume axis intercept of the PRSW relationship (not the slope) may be a better indicator of the severity of contractile dysfunction.
2. dP/dt Max vs. EDV: a linear regression of the maximum derivative of pressure to end diastolic volume. The slope generated is also used to address changes in contractility. In general, a steeper slope suggest a more responsive change in contractility, while a shallow slope suggests a poor response to load change.
3. PVA vs. EDV: The Pressure-volume area (PVA) represents the total mechanical energy generated by ventricular contraction. This is equal to the sum of the stroke work (SW), encompassed within the PV loop, and the elastic potential energy (PE) (PVA = PE + SW). Pressure-Volume Area vs. End diastolic volume therefore represents a linear regression of mechanical energy to volume during load change: a steeper slope suggests a more responsive, healthier heart, while a shallow slope suggests contractile dysfunction.



Maximum dP/dt vs End Diastolic Volume



Pressure Volme Area vs End Diastolic Volume

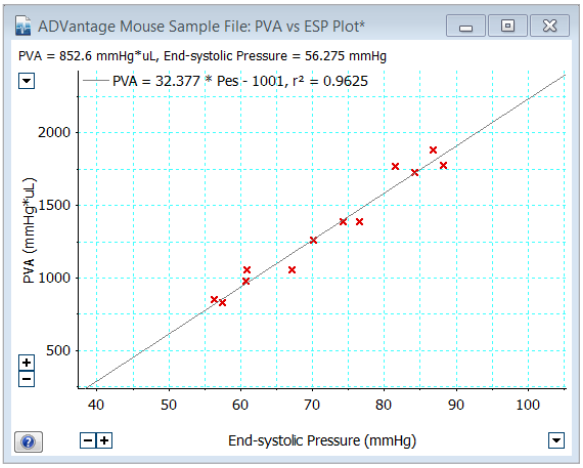
Analyzing PV Loop Data in LabChart Cont.

4. PVA vs. ESP: Pressure-Volume Area vs. End systolic Pressure represents a linear regression of mechanical energy to peak pressure during load change: a steeper slope suggest a more responsive healthier heart, while a shallow slope suggests contractile dysfunction.

ANALYSIS FUNCTIONS

Reselect Analyzed Data, Clear Analysis, and Analyze Selection are used to register, clear, or reselect (if changes are made but you wish to revert to the original sample of data points) data of interest.

Highlight data to be analyzed and select the appropriate analysis option. Choose from Baseline or Occlusion analysis based on the type of data.



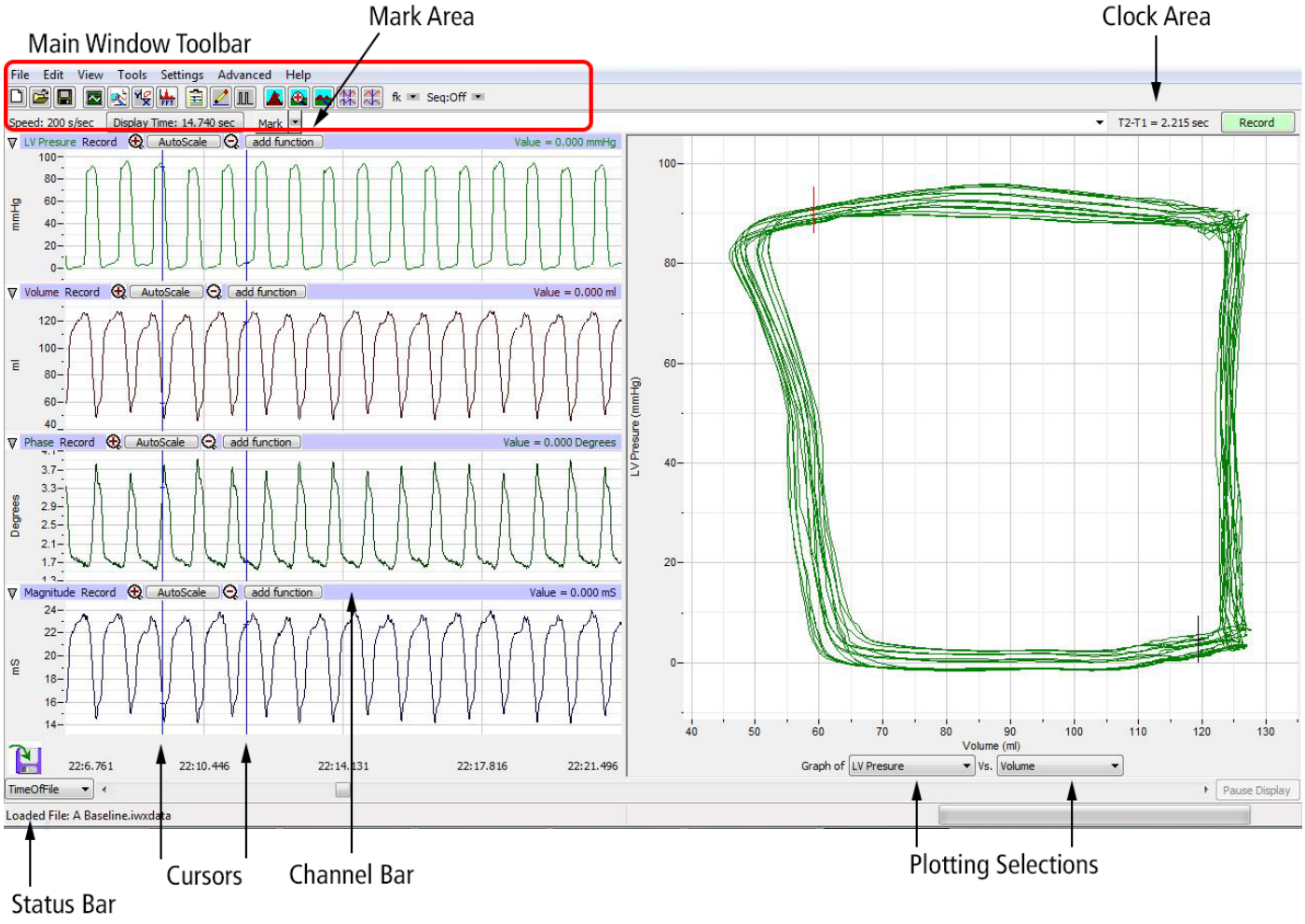
Pressure Volume Area vs End Systolic Pressure

Analyzing PV Loop Data in LabScribe2

The following document outlines suggested steps for analyzing PV loop data in LabScribe2 software when used in conjunction with the ADV500 PV system. Every experiment is different and may require different settings or the use of different software features. Please contact iWorx directly for additional information about all the available features or any software functionality issues.

For the purpose of this exercise, a sample file recorded from a healthy pig using the ADV500 PV system will be used. Only raw data was recorded at the time of the experiment, therefore, this example does not show hemodynamic measurements as calculated channels over time. Remember, that these channels can be created during analysis if required to show trends over the course of the experiment.

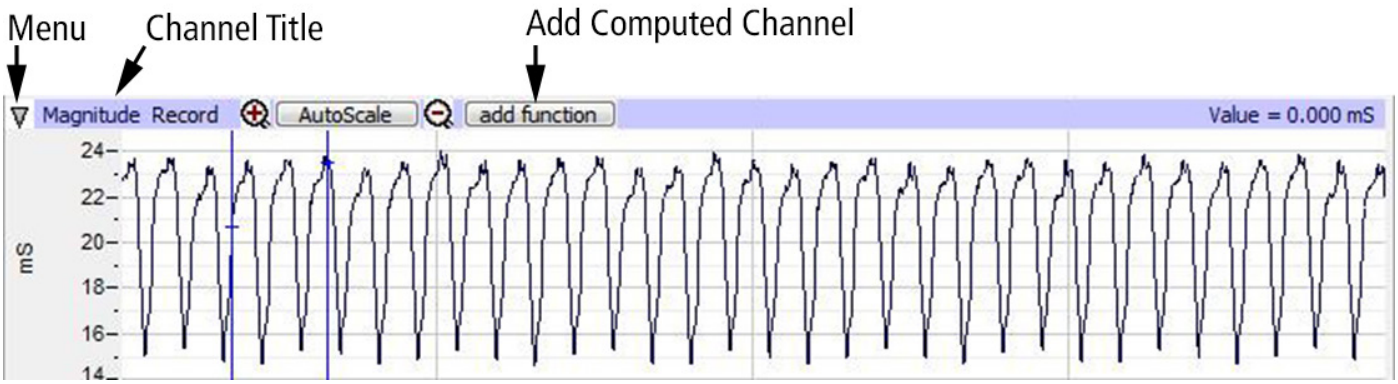
Basic Labscribe2 Functions for PV Loops



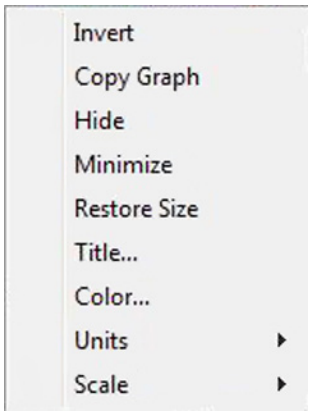
Full screen view of the pig Left Ventricle PV data collected using the ADV500 PV System. There are three channels of raw data: Pressure (mmHg), Phase (degree°) and Magnitude (µS or mS) and one calculated channel: Volume (ml). The XY view of Pressure-Volume Loops are on the right.

Analyzing PV Loop Data in LabScribe2 Cont.

The Main Window Toolbar features icons for quick access to key features that help with data manipulation and analysis.



Each channel has its own control located in the channel bar immediately above channel's data. The channel bar allows for zooming in and out, autoscaling the amplitude of recorded data, adding function to the channel, changing its title, etc.



The channel menu triangle contains functions specific to channel. The channel menu can also be accessed by right-clicking anywhere in the channel.

CHANNEL MENU

Important channel functions for data analysis include:

- Invert: Inverts the trace
- Title: Opens a dialog allowing a change to the title of the channel
- Color: Opens color palette allowing a change to the color of the channel trace
- Units: Displays unit conversion option
- Scale: Displays the option to control the vertical axis of the channel

Analyzing PV Loop Data in LabScribe2 Cont.

USING & MOVING CURSORS

The value of the single cursor or the difference between the two cursors is displayed in the Clock Area.

During data analysis, cursors can be moved by placing the mouse over the cursor clicking and dragging it to the right or left. The cursor on the left is always cursor number 1, while the cursor on the right is number 2. If you move cursor number 2 to the left past cursor 1 it becomes the new cursor number 1.

In the two cursor mode, the cursors can be locked at a set interval to allow for viewing a constant amount of data. To lock separation distance, position the two cursors at the desired separation and choose "Tools > Lock Cursor Separation" To unlock the distance click "Lock Cursor Separation" a second time.

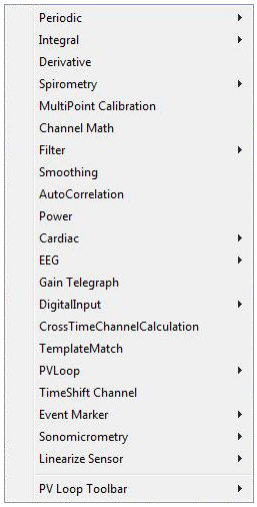
MARKING A CHANNEL

Click on Single Cursor Icon in the Toolbar. Position the cursor on the file where the mark should be positioned. Then type text associated with this mark into the Marks area and hit enter. To later edit or delete the mark, access the mark dialog from View menu or by clicking on the Marks Icon on the Toolbar. Marks are valuable for keeping track of key events but can also be used to make note of system variables or experimental conditions.

In addition to marking static data, you can also make Marks during live data collection. To accomplish this, simply type your intended information in the Mark area (as described above) and hit Enter. A mark will be time-stamped at the moment that you hit Enter, allowing for easy data review following protocol completion.

Addition of Computed Function Channel add function

The addition of computed functional channels can aid in analysis by providing important information e.g. adding the Derivative from the raw Pressure channel (dP/dt channel). Other useful computed functional channels include Integral, Channel Math, Filter and Smoothing. To add a functional channel to the current channel click on [add function].



Add function menu

DERIVATIVES

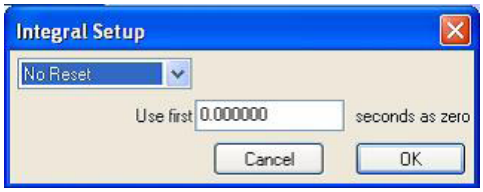
The Derivative function calculates the derivative (slope) around each point in the raw data, and then displays it on the calculated channel. On the new derivative channel, the units are changed to the units of the raw data channel/second. Higher order derivatives can be calculated by applying a Derivative function to a Derivative channel.

INTEGRALS

An integral is the area under a curve. In pressure-volume data analysis you mostly utilize the integral calculating channel for performing pressure wave analysis or calculating the integral of a flow waveform from a Transonic Flowprobe to obtain stroke volume (SV). There are four types of integrals; the two most common ones are Standard and Absolute.

Standard: Includes all data points in the calculation.

Absolute: The Absolute Value of the Integral, as the name implies, makes all values of the integral positive and plots the running total. Data points with values above zero make the integral larger, those with values less than zero make the integral smaller. To successfully calculate the integral, the location of the zero-line or baseline needs to be established.



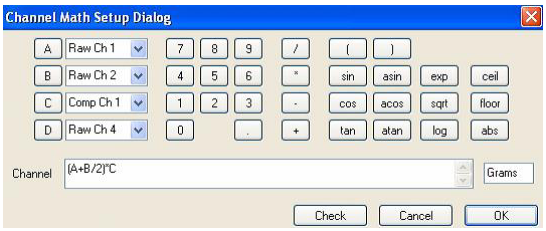
Setting the baseline for use with integral

Analyzing PV Loop Data in LabScribe2 Cont.

Setting the baseline of the raw data channel to zero is important because this function defines the difference between positive and negative areas of the recording. If the baseline of the raw data record is in the positive range of amplitudes, then the integral will have a positive slope, even though no signal is present. Conversely, if the baseline of the raw data is in the negative range, the integral will have a negative slope.

CHANNEL MATH

The Channel Math function applies a user-defined function to up to four data channels and displays the output in a computed channel. Each of four variables (A, B, C and D) can be associated with a user-selected data channel.



Channel Math allows for custom functions based on up to four channels

FILTERING

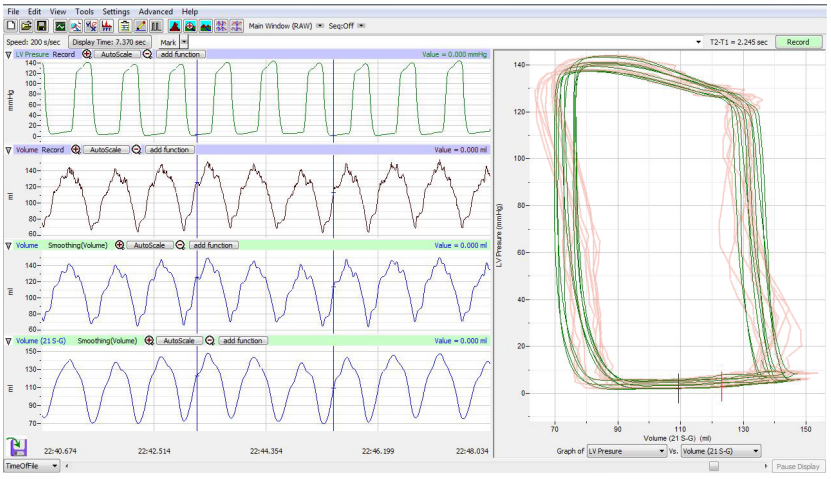
A digital filter can be applied to any channel in real time or to previously recorded data. LabScribe2 uses a FIR (Finite Impulse Response) filter. There are various windowing functions that can be used for setting up an FIR filter. The Hamming window (default) is appropriate for most applications. In addition to the Hamming function, LabScribe2 also provides Rectangular, Bartlett, Hanning, Blackman and Blackman-Harris windowing functions.

For the data analysis of pressure-volume data, use a Smoothing function rather than data filtering to prevent loss of the pressure high-fidelity signal. It has been established by researchers that in order to accurately recreate a blood pressure waveform, the sensing system must be able to accurately measure up to the 20th harmonic of the observed waveform (1). Furthermore, Dr. Grossman reports in his work the accuracy of capturing and reporting the pressure signal: “If components of a particular frequency range are either suppressed or exaggerated by the transducer system, the resulting signals will be greatly distorted (2).”

If you choose to filter your data, on the channel of interest, click on [add function] then select “Periodic > Frequency”. A Frequency channel in Hz will be created. Determine the highest frequency and then multiply by 20 to get the 20th harmonic. That value will become the “High Cutoff” value for the FIR filter. To apply the filter click on [add function] select “Filter > FIR Filter”.

SMOOTHING

Smoothing is used to remove noise that is uniform across all frequencies. LabScribe2 has the ability to smooth the data in order to reveal significant features of your data. There are two different algorithms to smooth the data; the Moving Average function does wide smoothing, while the Savitzky-Golay seeks to preserve shapes of peaks.



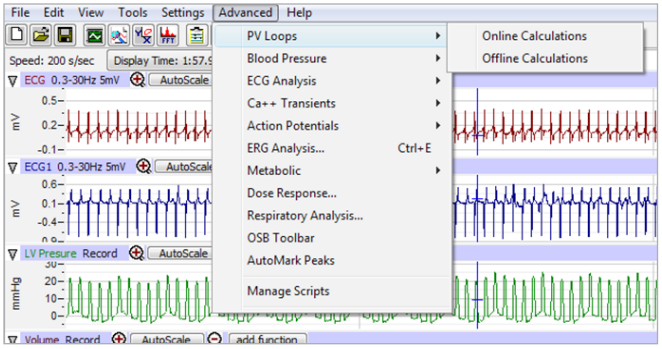
The first channel is raw pressure data. The second channel is unmodified volume data. The third channel is volume data smoothed by Savitsky-Golay second order polynomial with 7 points of data on each side. The fourth channel is volume data smoothed by Savitsky-Golay second order polynomial with 21 points of data on each side. The red PV loops are created from the un-smoothed data while the green loops are from the 21 points smoothed data.

Analyzing PV Loop Data in LabScribe2 Cont.

SMOOTHING CONT.

The Savitzky-Golay method essentially performs a local polynomial regression to determine the smoothed value for each data point. For analysis of pressure-volume this method is superior to Moving Average because it tends to preserve features of the data such as peak height and width, which are often diminished by adjacent averaging.

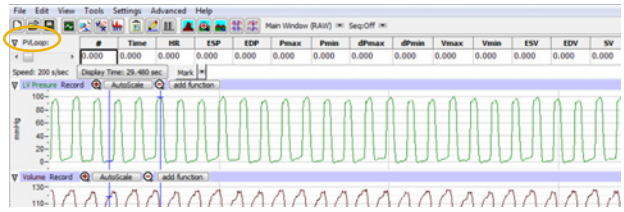
When using the Savitsky-Golay smoothing function, it is best to start with a smaller number of points on each side and then increase the number of points later after assessing how the smoothing impacts the data to prevent data distortion from over smoothing.



Select PV Loop Online or Offline Calculations

Specific PV Loop Functions

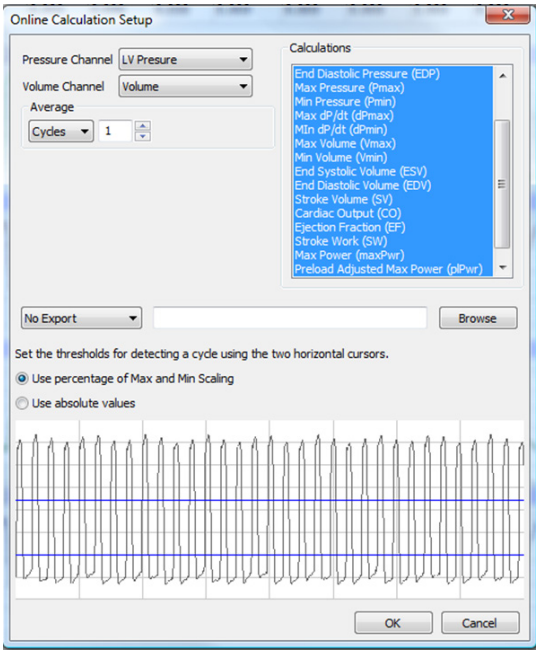
Analysis specific to PV loops is started by choosing the "Advanced > PV Loops", then choosing one of two options: Online Calculations and Offline Calculations.



Open the PV Loop Online Calculations Setup Menu by clicking the triangle in the corner.

ONLINE CALCULATIONS

Choose Online Calculations from the PV Loops submenu. Click on the triangle in the upper left corner to open the PV Loop Online Calculations Setup window. Select the appropriate Pressure Channel and Volume Channel for the loops. Set Average Cycles to something other than zero, otherwise the loop will never refresh. Set the threshold for detecting cycles to "Use percentage of Max and Min scaling" and adjust the threshold bars to include acceptable pressures. Keep in mind the impact of IVC occlusion (reduced pressure) when setting the threshold to ensure all cycles are included.



PV Loop Online Calculations Setup Menu

OFFLINE CALCULATIONS

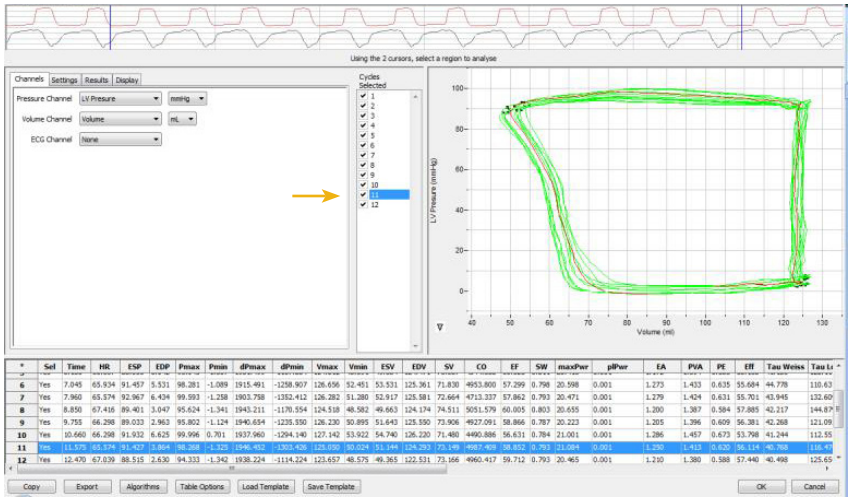
Choose Offline Calculations from the PV Loops submenu to open the PV Loop Calculations Dialog. The panels of this dialog can be re-sized by moving the mouse cursor over the boundaries until a double headed arrow appears, and dragging the boundaries to re-size the panels.

LabScribe2 can calculate virtually every PV Loop derived parameter (Steady-state or post-occlusion condition) of cardiac function. XY graphs display relationships among the parameters. All calculations can be exported or copied to MS Excel or other spreadsheets for further analysis. The graphs can all be copied as images to include in presentations or manuscripts. When the Offline calculations is open, 4 tab panels become available: Channels, Setting, Results and Display.

Analyzing PV Loop Data in LabScribe2 Cont.

CHANNELS

The Channels tab enables the selection of the channels that will be displayed in the XY graph. To create PV loops select the Pressure channel and Volume channel along with the appropriate units. Once the Pressure and Volume channels are designated, the PV Loops will be displayed in the XY graph area for all the cycles in the selection. Define the region of interest by adjusting the two cursors in the channel display area at the top of the PV Loops Calculations Dialog. This will select the amount of PV loops (in this case 12).



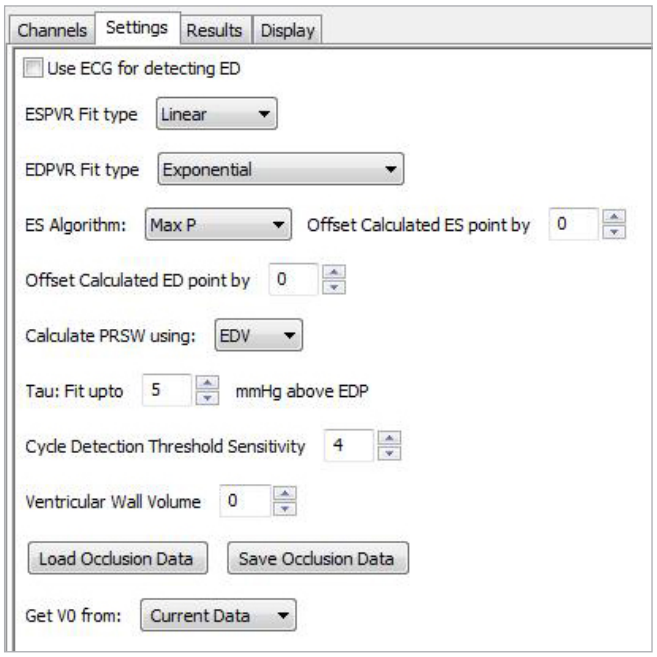
Channels tab for selecting the data channels used to create the PV loops. Use the Cycles Selected menu to include or exclude specific loops.

If you wish to add more PV loop cycles than the amount available in the top channel, you have to go back to the Main Window and use the double display time icon to increase the amount of PV loop data.

The highlighted cycle in the Cycles Selected window is shown in red, while all other selected cycles are displayed in green. Parameters related to each cycle are shown in the table at the bottom. Cycles can be deselected (or selected) by clicking on the check box to the left of the cycle number. This allows for identifying individual loops (individual cycles) for inclusion or exclusion from the analysis.

SETTINGS

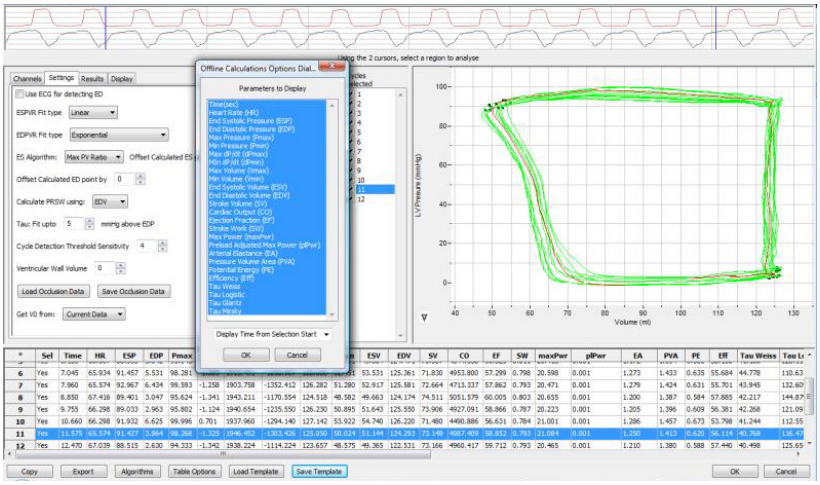
- When recording using ECG channel, check whether you wish to use an ECG for the detection of end diastole (ED).
- For OCCLUSION condition, please choose whether a linear or an exponential fit should be applied to the End-Systolic Pressure-Volume Relationship (ESPVR) data, using the ESPVR Fit Type drop-down box. In rodents, linear is generally used; in larger animals, compare both linear and exponential fit. Please review appropriate literature in order to select ESPVR fit type.
- For OCCLUSION condition, leave EDPVR fit type as exponential. This relationship is based on literature citation (3). If pressures fall below zero, a message will appear requesting that the data be offset in the positive direction in order to perform the calculation.



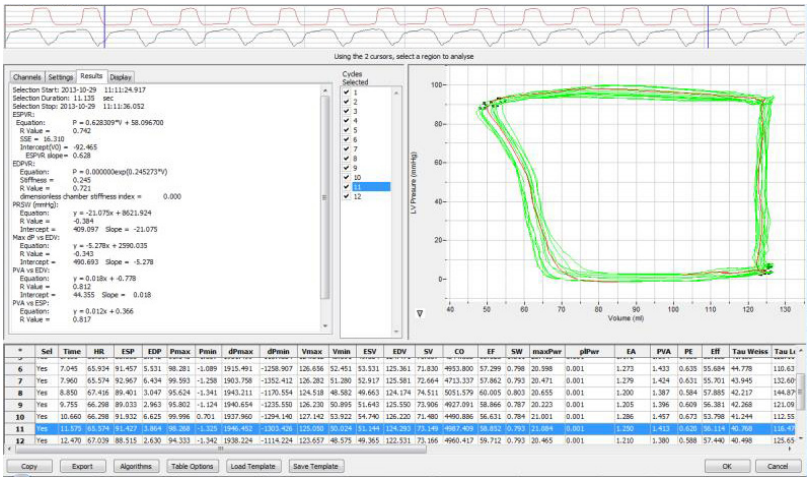
Settings tab for selecting the methods for calculating key parameters

Analyzing PV Loop Data in LabScribe2 Cont.

- Choose the value in each cycle that should be used to compute end-systole (ES), and how far to offset ES from the specified value.
- For OCCLUSION condition, by default, the maximum Pressure to Volume ratio is used to identify ES. When calculating PRSW (Preload Recruitable Stroke Work), it is calculated by using detection of EDV rather than V max. If the isovolumic contraction period of the PV loop is deformed or, in case of RV-PV loops, select Vmax to reanalyze.
- Enter the stop value to be included in the best fit line used for the determination of isovolumic relaxation time (IVRT) Tau. Tau is the isovolumic time constant and is calculated using the line from dPmin to this user-defined point. Leave the value of 5 mmHg as a user defined point for the beginning of analysis. For more info about Tau please see "Understanding Lusitropy" on page 20.
- For Cycle detection threshold sensitivity and Ventricular Wall Volume, leave the number of cycles that should be used to determine the Cycle Detection Threshold Sensitivity with default values.
- In case you wish to use PV loop(s) superimposed on each other for comparison ,please select Save Occlusion data tab. Select File name and iWorx Occlusion file (.iwxocc) format. This superimposed data can also be used to compare PV loops during Steady state conditions or to compare multiple vs single loops.



Click [Table Options] to adjust which parameters are displayed



Results tab to view the equations used for calculating key parameters

RESULTS

The equations defining ESPVR, EDPVR, PRSW, Max dP vs. EDV, PVA vs. EDV, and PVA vs. ESP are displayed, as is Emax. By clicking on the Algorithm button, parameters are detailed including the description/or literature citation. Parameters that are displayed can be easily copied using the copy button. When you select copy, parameters are copied for both occlusion and steady state, although only occlusion values are displayed. When copied into MS Excel or another program, they can be easily post-processed.

Analyzing PV Loop Data in LabScribe2 Cont.

DISPLAY

Clicking on a graph color allows for selection of a basic color or for creating a custom color for each type of selection. When it is necessary to hide a selection, as in the case of PVR lines for description of steady state PV loops, the color can be set to white.

Other display parameters that will be shown on PV loop graph include ES location, ED location, ESPVR, EDPVR, Ees vs. Ea, Tau start, Tau stop, and Average loop. When selected, parameters will appear on the PV loop graph.

Display tab for selecting the parameters visible on the graph and their colors.

REFERENCES

(1) Bernard J Gersh, Clive E.W. Hahn and Cedric Prys-Roberts: "Physical Criteria for Measurement of Left Ventricular Pressure and its First Derivative." Cardiovascular Research, 1971, 5, 32-40

(2) Dr. William Grossman, Grossman's Cardiac Catheterization, Angiography, & Intervention, 7th Edition: 2006 Lippincott Williams & Wilkins. pg 134

(3) Burkhoff D, Mirsky I, Suga H. "Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers." Am J Physiol Heart Circ Physiol. 2005 Aug;289(2):H501-12

Post-Calculating Admittance Volume in LabScribe2

The Scisense ADV500 system offers a method for deriving ventricular volume called admittance. While the ADV500 system derives true ventricular volume in real-time, there are certain circumstances where the researcher may wish to manually calculate volume using the admittance method post-experiment. The benefit of doing this is that it offers the researcher full control of all of the variables that influence the resulting volume. Referring to Wei's equation, these variables are: Stroke Volume (SV), Blood Resistivity (ρ), Heart Type (σ/ε), Measuring Electrode Distance (L), Blood Conductance at End-Systole ($G_{b\text{-}ES}$) and Blood Conductance at End-Diastole ($G_{b\text{-}ED}$).

The following outlines how to use LabScribe2 software from iWorx to analyze the raw data traces of Magnitude, Phase, and Pressure to manually calculate volume using the admittance process and Wei's equation.

First select a stable baseline data set.

On the Magnitude Channel select "Add Function > PVLoop > Blood Conductance."

Wei's Equation

$$Vol = \frac{1}{(1-\frac{G_b}{\gamma})}\rho L^2(G_b)$$

VARIABLES

ρ = blood resistivity

L = measuring electrode distance

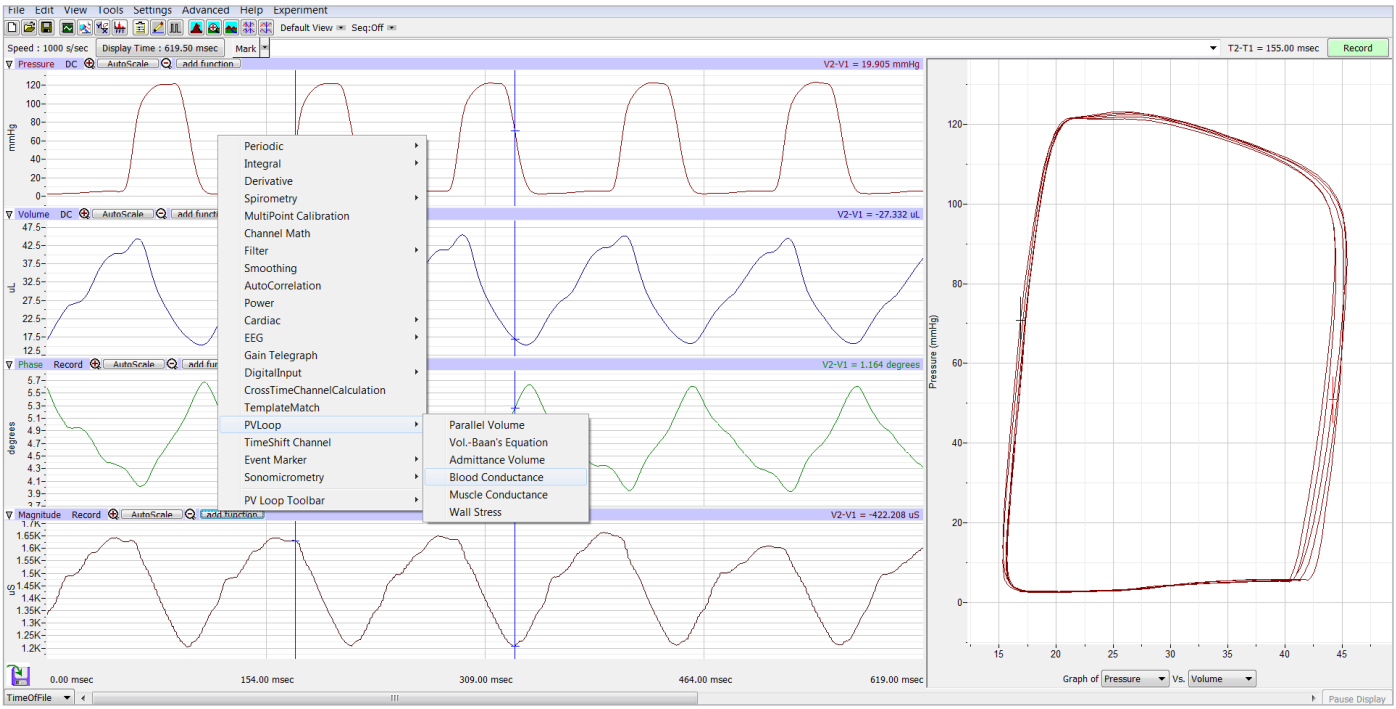
G_b = measured Blood conductance (separated from parallel/muscle conductance using admittance phase measurement)

$$\gamma = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

$$a = SV - \rho L^2(G_{b-ED} - G_{b-ES})$$

$$b = -SV \cdot (G_{b-ED} + G_{b-ES})$$

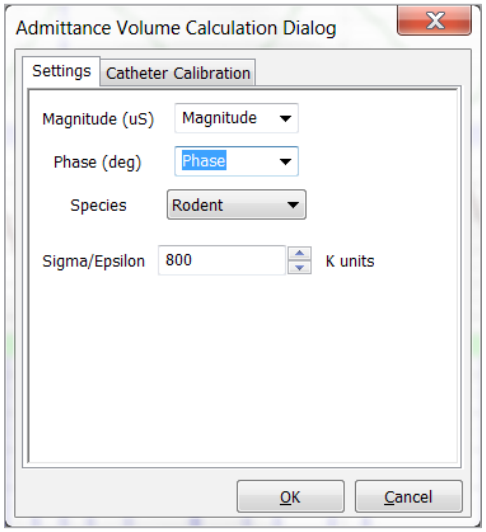
$$c = SV \cdot G_{b-ED} \cdot G_{b-ES}$$



Baseline PV data set. Use add function button on the magnitude channel to add Blood Conductance channel

Post-Calculating Admittance Volume in LabScribe2 Cont.

After selecting "Blood Conductance" a dialog box will appear (pictured right). Complete the "Settings" for Magnitude, Phase, Species and Sigma/Epsilon, also referred to as 'Heart Type' in current ADV500/ADVantage 5.0 systems. In this example, the standard for healthy tissue was used (S/E = 800,000 units). After selecting the "OK" button on the dialog box, a new channel for blood conductance will appear.

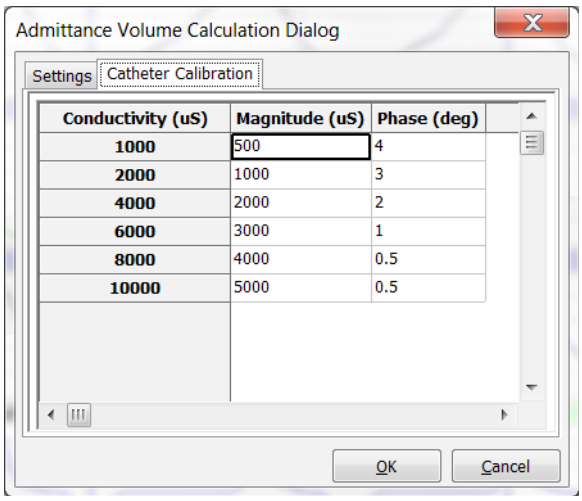


Admittance Volume Calculation dialog box

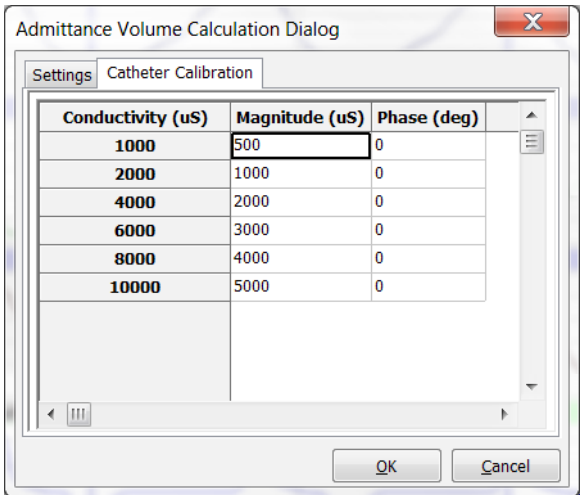
Important: Catheter Calibration Tab

This section allows the researcher to account for any internal capacitance inherent to the Catheter itself.

- For data sets collected with the ADVantage PV System (Version 3.0 – 4.52), you can access this table of information on the ADVantage Control Unit by connecting the Catheter that was used to acquire the data and navigating to the "Catheter Information" option (see manual for specific menu location). Entering the specific values for that Catheter are ideal. However, if this information is inaccessible, resort to the defaults provided (example below on left).
- For data sets collected with the ADVantage/ADV500 PV System (Version 5.0 or greater), the internal capacitance of the Catheter is automatically removed from the phase signal before it is output as an analog signal to your data acquisition system. Therefore, there is no additional correction for internal capacitance required. To negate this process in LabScribe2, enter a "0" in each cell in the Phase column (example below on right)



Catheter Calibration for ADVantage Version 3.0-4.52



Catheter Calibration for ADVantage Version 5.0+

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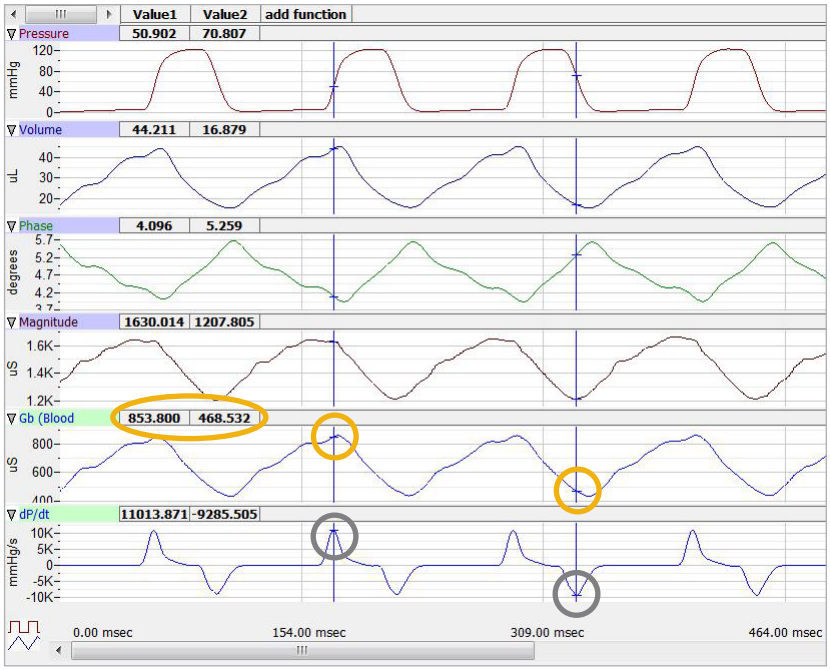
Post-Calculating Admittance Volume in LabScribe2 Cont.

On the Pressure Channel, select "Add Function > Derivative." After selecting "Derivative" a new channel will appear for dP/dt. This channel will be used to accurately locate the end-diastolic and end-systolic time points.

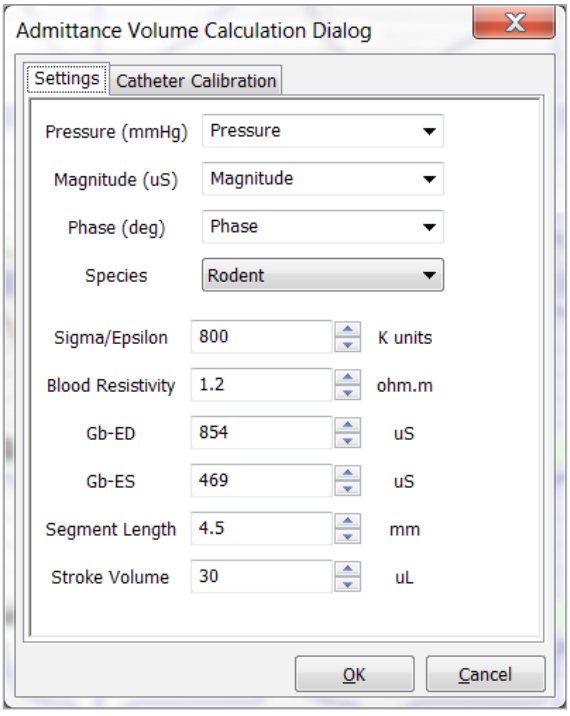
Change to Analysis View by selecting "View > Analysis" or by selecting the Analysis View icon. Use the vertical cursors to highlight maximum and minimum dP/dt points and corresponding measurements $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$, respectively. Make note of the measured $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ values and return to the Main Window view.

On the Magnitude Channel select "Add Function > PVLoop > Admittance Volume".

After selecting "Admittance Volume" a dialog box will appear (pictured right). Complete the Settings for Pressure, Magnitude, Phase, Species, "Sigma/ Epsilon" (also referred to as "Heart Type" in ADV500/ADVantage 5.0 system), Blood Resistivity, $G_{b\text{-}ED}$, $G_{b\text{-}ES}$, Segment Length (Measuring Electrode Distance), and Reference Stroke Volume. Address Catheter calibration tab as previously described above.



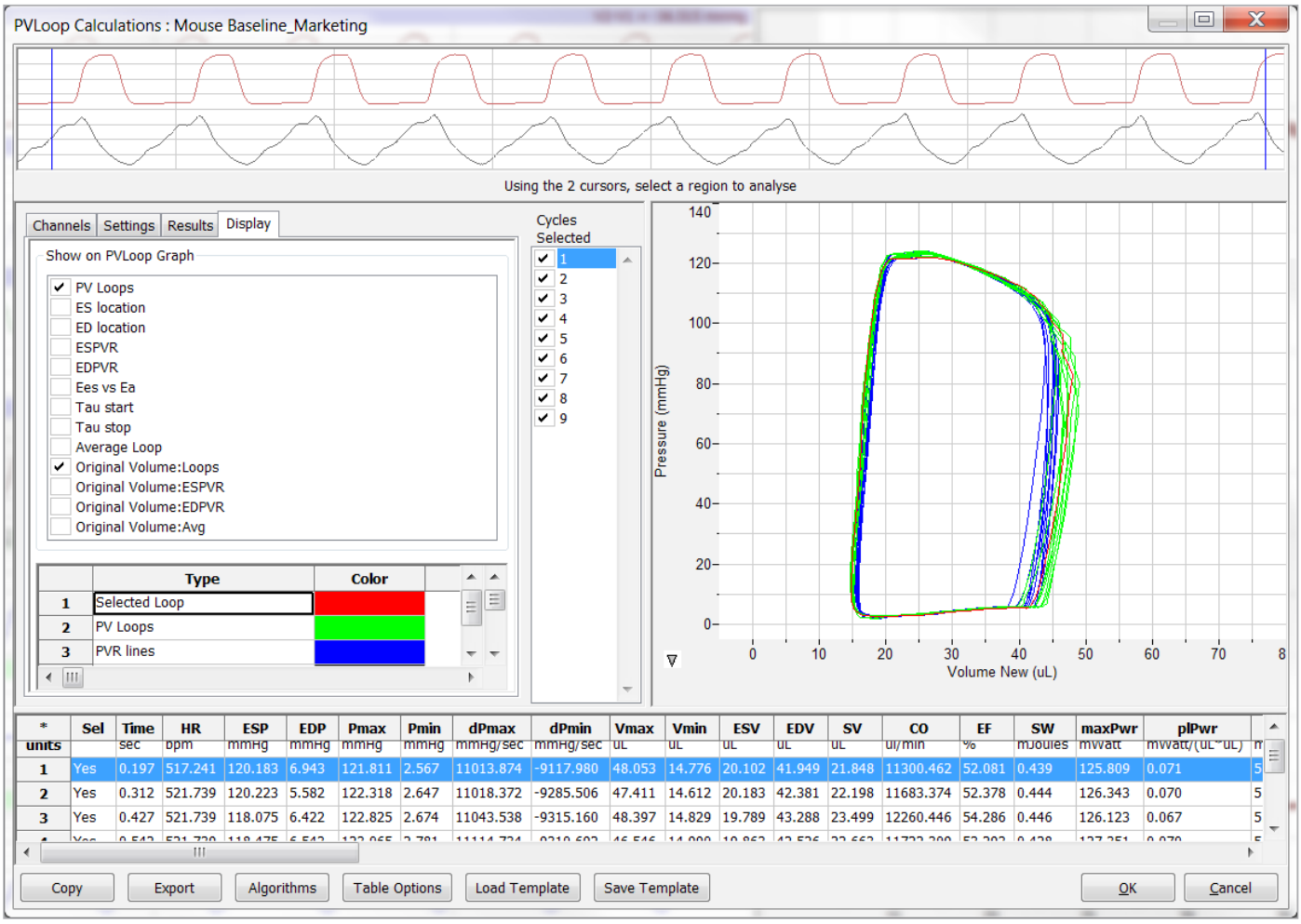
dP/dt min and max points corresponding to $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$, respectively



$G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ are input into admittance volume calculation

Post-Calculating Admittance Volume in LabScribe2 Cont.

After selecting "OK" on the dialog box a new channel for Admittance Volume will be populated. Rename the channel as desired (ex. Volume New). To quantify the difference between the new volume derivation and the original recorded at the bench, use the "Offline PV Loop" analysis functions. You can superimpose the data sets and see if the alterations in coefficients for Wei's equation (such as SV reference or blood resistivity) derive a significantly different volume. Outside of the coefficients, the post calculated volume theoretically yields the most accurate volume, as the measurement of $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ are specific to that moment of the data file (this is another valuable use of the post calculation process in the event an accurate baseline scan was not performed during live data collection).



*	Sel	Time	HR	ESP	EDP	Pmax	Pmin	dPmax	dPmin	Vmax	Vmin	ESV	EDV	SV	CO	EF	SW	maxPwr	plPwr
units		sec	bpm	mmHg	mmHg	mmHg	mmHg	mmHg/sec	mmHg/sec	uL	uL	uL	uL	uL	ul/min	%	mJoules	mWatt	mWatt/(uL^uL)
1	Yes	0.197	517.241	120.183	6.943	121.811	2.567	11013.874	-9117.980	48.053	14.776	20.102	41.949	21.848	11300.462	52.081	0.439	125.809	0.071
2	Yes	0.312	521.739	120.223	5.582	122.318	2.647	11018.372	-9285.506	47.411	14.612	20.183	42.381	22.198	11683.374	52.378	0.444	126.343	0.070
3	Yes	0.427	521.739	118.075	6.422	122.825	2.674	11043.538	-9315.160	48.397	14.829	19.789	43.288	23.499	12260.446	54.286	0.446	126.123	0.067

Superimposed data sets (Original Volume is Blue and New Volume is Green). Note: No visible difference is observed in the volume measurements because the original volume was derived in real-time using the exact same volume coefficients for Wei's equation and an accurate baseline scan was performed within this range of data.

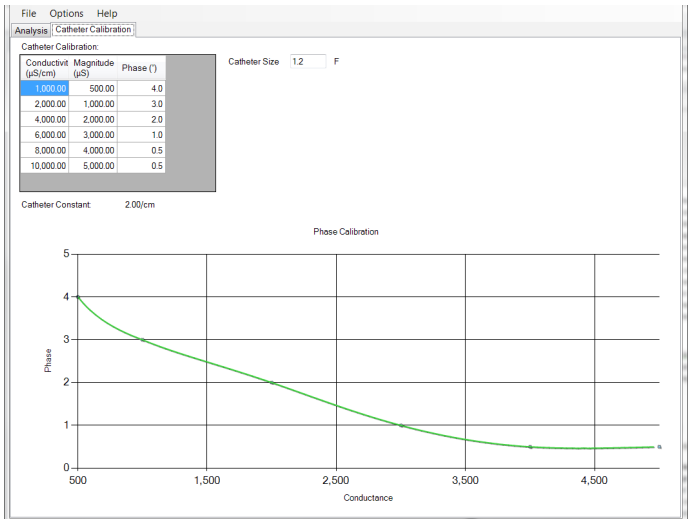
Post-Calculating Admittance Volume in AdVol

The Scisense ADV500 system offers a method for deriving ventricular volume called admittance. While the ADV500 system derives true ventricular volume in real-time, there are certain circumstances where the researcher may wish to manually calculate volume using the admittance method post-experiment. The benefit of doing this is that it offers the researcher full control of all of the variables that influence the resulting volume. Referring to Wei's equation, these variables are: Stroke Volume (SV), Blood Resistivity (ρ), Heart Type (σ/ε), Measuring Electrode Distance (L), Blood Conductance at End-Systole ($G_{b\text{-}ES}$) and Blood Conductance at End-Diastole ($G_{b\text{-}ED}$).

The following outlines how to use AdVol software from Transonic to analyze the raw data traces of Magnitude, Phase, and Pressure to manually calculate volume using the admittance process and Wei's equation. After generating the new volume, pressure and volume data can be saved in text format and imported into a PV analysis software.

Upon opening AdVol software you will be prompted to select which version of the ADVantage/ADV500 PV system was used to acquire the data file of interest.

By selecting "ADVantage 4.x", the user will be presented with the option to input Catheter calibration values on the "Catheter Calibration" tab (see below). You can access this table of information on the ADVantage Control Unit by connecting the Catheter that was used to acquire the data and navigating to the "Catheter information" option (see manual for specific menu location). Entering the specific values for the catheter used are ideal. However, if this information is not available simply resort to the defaults provided. When selecting "ADVantage 5.x/ADV500", the "Catheter Calibration" tab appears blank.



Catheter calibration for ADVantage 4.x systems

Wei's Equation

$$Vol = \frac{1}{(1-\frac{G_b}{\gamma})}\rho L^2(G_b)$$

VARIABLES

ρ = blood resistivity

L = measuring electrode distance

G_b = measured Blood conductance (separated from parallel/muscle conductance using admittance phase measurement)

$$\gamma = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

$$a = SV - \rho L^2(G_{b-ED} - G_{b-ES})$$

$$b = -SV \cdot (G_{b-ED} + G_{b-ES})$$

$$c = SV \cdot G_{b-ED} \cdot G_{b-ES}$$

WHY IS THE SYSTEM VERSION IMPORTANT?

Original versions of the ADVantage PV system (3.0-4.52) provide a phase output signal which includes the intrinsic capacitive contribution of the PV catheter itself, while newer versions of the ADVantage/ADV500 PV system (5.0 +) do not. Therefore, if the data set of interest was collected with an earlier version of the system it is appropriate to remove this 'internal Catheter capacitance' contribution from the phase signal before moving forward with the admittance volumetry process.

Post-Calculating Admittance Volume in AdVol Cont.

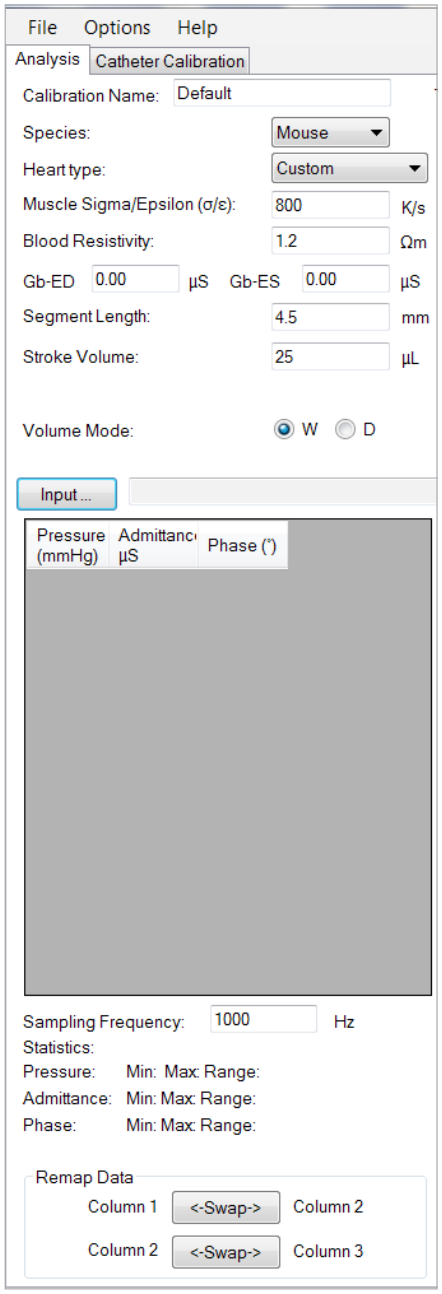
After selecting the correct data version, the main window will populate. In the left margin, all features and controls are available for loading a sample file, addressing admittance volume variables, and applying standards or saved settings.

- Define a calibration name in the event you wish to use the same settings for a series of data sets (ie. Control Mouse_Group 1).
- Select the appropriate Species from the Drop Down menu (Mouse, Rat, Rabbit, Large). Note: the term "Large" is appropriate for all animal data sets collected with 5F and 7F PV Catheters (ex. Dog, Pig, Sheep).
- Select the correct 'Heart Type' for the data file of interest, or use 'Custom' to define a specific Muscle Sigma/Epsilon value.
  - REMINDER: Standard values are 800 K/s for healthy heart tissue, and 900 K/s for hearts with any degree of infarct.
- Input values for Blood Resistivity; Segment Length, and Stroke Volume Reference value.
- Next, select the mode by which you wish to run the admittance volume calculation. "W" mode is the standard method which relies on the published and validated process including Wei's equation. "D" mode is a simplified process that does not take into account the full Phase wave information. This should only be used as a last resort if the recorded phase information is corrupt or other circumstances affected proper recording of Phase. For more information, please refer to the ADVantage/ADV500 User Manual.
- Finally, type in the 'Sampling Frequency' at which the original data was recorded. Typical rodent data files are recorded at 1000 Hz, while larger animal data files are recorded between 200 and 500 Hz. It is imperative that the exact sampling value used to record the original data is entered or the data file will not map properly when imported.

Once all settings are addressed, click "Input". A dialogue box will appear allowing you to select a file of interest. The following file formats are acceptable: Text (.txt), Comma Separated (.csv), Tab Separated (.tab), Ponemah (.ascii).

After selecting a file the data table columns for Pressure, Admittance/ Magnitude, and Phase will auto-populate. AdVol should recognize the data values that are correct for each column. However, if they load incorrectly the "Swap" buttons below the table can be used to shift the data values to the correct place.

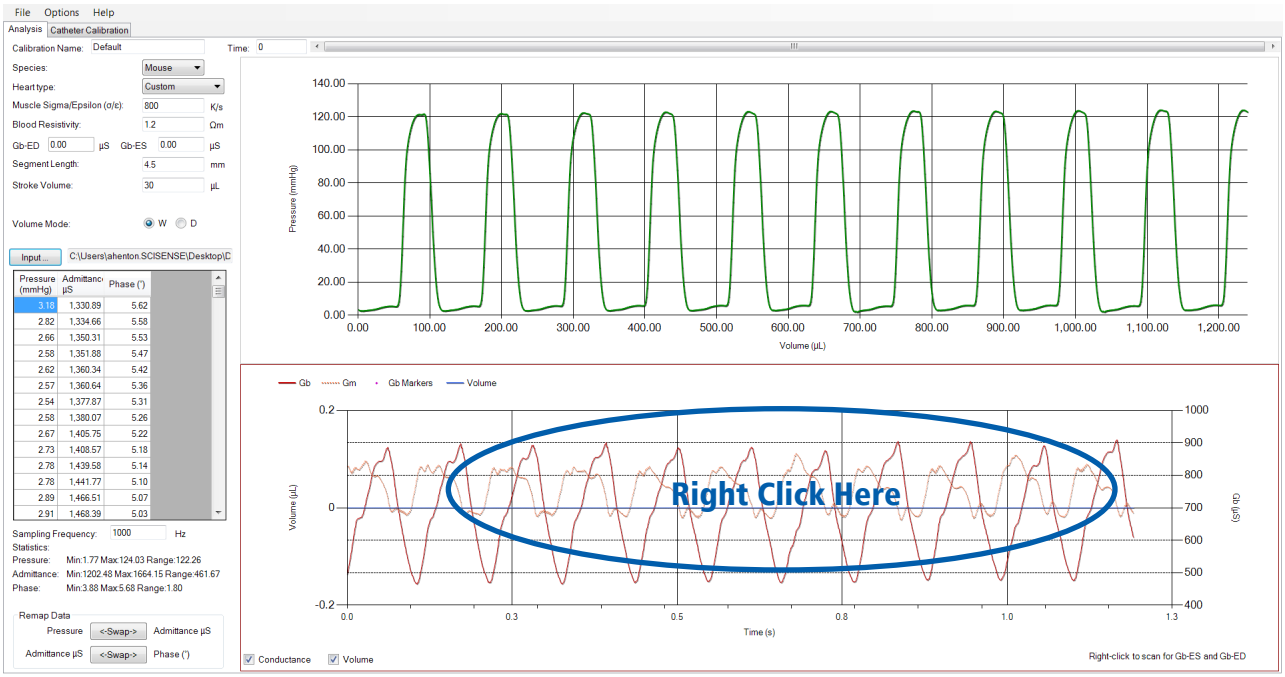
Once the data is correctly mapped and all variables are confirmed, the wave forms should be interpreted in the main window in order to sample $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ values.



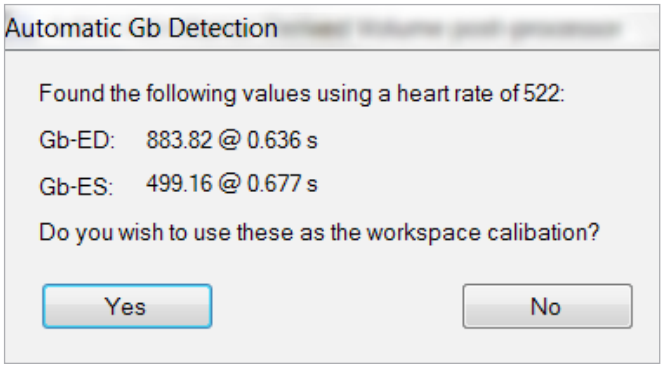
Admittance volume variables are required for analysis

Post-Calculating Admittance Volume in AdVol Cont.

In the main window area, right click on the lower graph to activate a "baseline scan:"



Perform a baseline scan by right clicking in the lower graph to automatically detect $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ values



Use the automatic detection values of $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$

The results of the scan will provide a measured value for $G_{b\text{-}ED}$, $G_{b\text{-}ES}$, and will show where on the wave trace these values were calculated. Remember: $G_{b\text{-}ED}$ should always be larger than $G_{b\text{-}ES}$, and both values should be positive. If the scan results in negative numbers or the values are outside of physiological range repeat the scan and double check that the data is properly mapped.

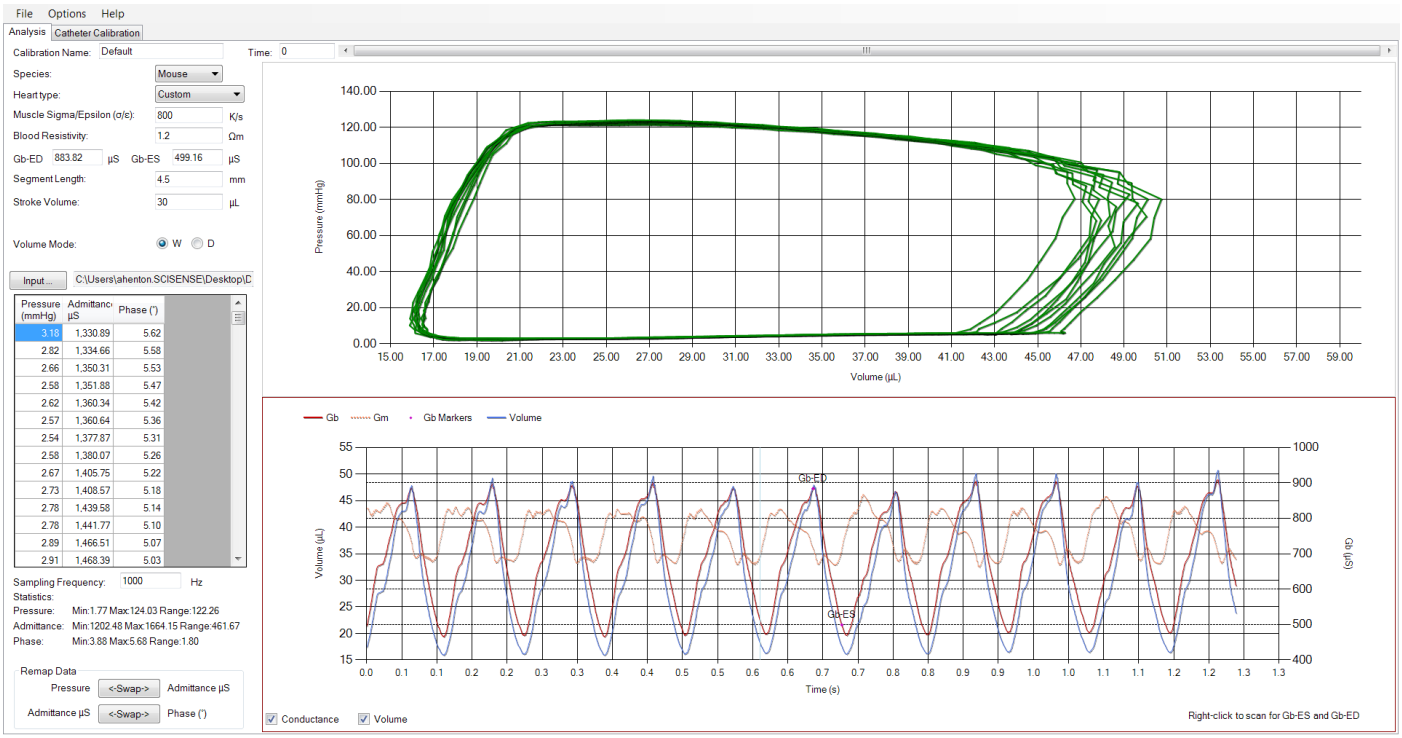
After clicking "Yes", the $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$ values will auto-populate in the left margin. Waveforms for both Blood Conductance and Volume will appear in the main window, and a pressure vs. volume graph will be created.

Post-Calculating Admittance Volume in AdVol Cont.

Check boxes are located in the bottom left corner of the main window allowing Conductance and/or Volume waveforms to be turned off. This assists in viewing one particular waveform at a time to aid in checking that the values are physiologically acceptable.

At this stage admittance volume variables can be altered, if needed. Simply type the values into the appropriate cell in the left margin. To ensure the effects of altering a variable are carried through to the volume derivation completely, it is recommended that another scan is performed for $G_{b\text{-}ED}$ and $G_{b\text{-}ES}$.

Once a final volume suitable for export is rendered, click "File > Save Data" to export. The saved file will include only Pressure and Volume data and will have the same sample frequency as the import file (ie. 1000 Hz). Data can be saved in the following formats: Text (.txt), Comma Separated (.csv), Tab Separated (.tab). These are common file formats that are accepted by most commercially available PV loop analysis software programs such as ADInstruments LabChart, EMKA IOX, Notocord Heme, and BioPac Acqknowledge.



Final calculated PV loop based on admittance values in the left side bar

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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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