T400-Series Surgical Protocol

Rat & Mice: Coronary Blood Flow: Isolated Perfused Heart Preparation: Langendorff and Working Heart Models

APPLICATION BASICS				
MODEL	MOUSE		RAT	
	FLOW RANGE (ml/min)	INLINE FLOWSENSOR	FLOW RANGE (ml/min)	INLINE FLOWSENSOR
Langendorff	2 – 8	ME1PXN ME2PXN	7 - 30	ME2PXN ME3PXN
Working Heart (2 Inline Sensors)	8 – 15		40 – 80	
Flowmeter	TS410 Tubing Module			
Calculated Cardiac Output = Coronary (Atrial Inflow) – Aortic Outflow				

Fig. 1: Inline Flowsensors: ME1PXN, ME2PXN and ME3PXN

FLOWSENSOR CALIBRATION	
SOLUTION	Krebs blood or other Physiological Buffer Solution (sample may be required for factory calibration)
TEMPERATURE	37 C°
FLOW RATE	Isolated heart applications require calibration in a custom low flow range for the flow rates indicated above that are typical for isolated heart preps. Higher flow rates may be requested for working heart preparations.
Note: Isolated heart applications require calibration in a custom low flow range for the flow rates indicated above that are typical for isolated heart preps. Higher flow rates may be requested for working heart preparations.	

Background

The first isolated perfused mammalian heart preparation was developed by Oscar Langendorff in the 1890's and continues to be a viable technique to study myocardial function today. Early discoveries in heart physiology originating from this technique include the roles of temperature, oxygen, calcium ions, the electrical activity during the cardiac cycle, the role of coronary circulation to deliver nutrient and oxygen rich blood to the heart and, importantly, that cardiac mechanical function is affected by changes in the coronary circulation.

Langendorff Method

This in vitro isolated organ technique allows the study of contractile force, heart rate, coronary resistance and other parameters of the heart under known physiological conditions without the neural and hormonal complications of an in vivo, whole animal experiment. In the Langendorff heart preparation, the heart is isolated from the animal, a cannula is inserted into the ascending aorta and the heart is perfused in a retrograde direction with blood or, more commonly, oxygenated nutrient rich crystalloid solution from a gravity fed reservoir. Pressure from the retrograde perfusion causes the aortic valve to close and forces the solution into the coronary circulation, draining via the coronary sinus into the right atrium. Contractions in the heart will continue in this state and various parameters can be measured.

There have been several advances in the apparatus and instrumentation to conduct these experiments, but the methodology remains fundamentally the same in the Langendorff preparation. The heart is perfused in a retrograde fashion in one of two modes: by constant pressure or constant flow.

Constant Pressure Mode

The perfusion of the heart is maintained at a constant pressure. Changes in resistance of the heart will result in fluctuations in the flow rate that are measured with a Transonic® Inline Flowsensor.

(Continued on next side.)

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THE MEASURE OF BETTER RESULTS.

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Application cont.

Constant Flow Mode

The perfusate is pushed through the heart at a constant flow rate. Changes in the resistance of the heart will result in fluctuations in pressure that can be monitored with a pressure transducer.

Additional parameters that can be measured in the isolated beating heart include:

- Left Ventricular Pressure via a balloon inside the ventricle equipped with a pressure transducer.
- Contractile Force via a force transducer attached to the apex of the heart.
- Electrical Activity is monitored via electrodes attached to the apex and atria.

Working Heart Method

The Langendorff isolated heart method was modified later by Neely et al to produce a model that would allow the study of the isolated heart under normal circulatory dynamics. In the working heart model, a second cannula is inserted into the pulmonary vein to perfuse the heart via the left atrium. The working heart pumps the fluid from the left ventricle out of the aorta under experimentally controlled preload (atrial pressure) and afterload (aortic resistance) conditions. This allows the measurement and calculation of additional parameters under the conditions of work. Flow in the atrial perfusion inflow cannula and aortic outflow cannula are measured with Transonic® Inline Flowsensors to calculate cardiac output (aortic flow plus coronary flow) and coronary flow (atrial inflow – aortic outflow). Coronary flow may be derived from the two Flowsensors or by collecting and weighing the coronary effluent. Other parameters that can be derived from the pressure and volume flow measurements are stroke volume, stroke work and vascular resistance.

Significance

Both the Langendorff isolated perfused heart and the working isolated heart methodologies have been used extensively in pharmacological and physiological studies to access ischemia and reperfusion injury with various pharmacological agents. This method remains popular because of the simplicity and ease of control, reproducibility and relatively low economic cost.

Common Donor Animals: rats, transgenic mice, guinea pig and rabbit

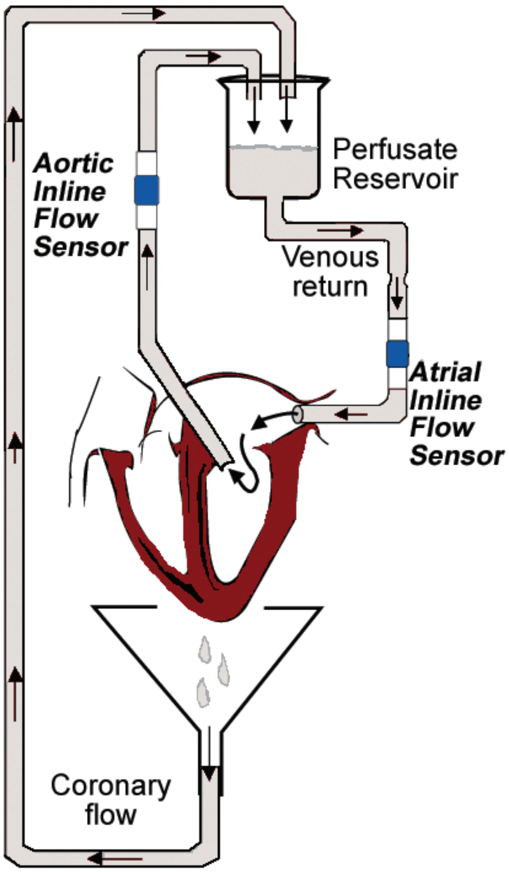

Fig. 2: Schematic of the working heart model of an isolated perfused heart preparation.

Langendorff Apparatus Sources:

Harvard Apparatus, Inc.

Hugo Sachs Electronik (see Harvard Appartaus)

Rattus (Kent Scientific)

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

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Preparation

Anesthetize the rat with pentobarbital (60 mg/kg IP). To prevent coagulation, administer heparin (1000 IU/kg) intravenously in the right femoral vein (See Anesthetic Guidelines RL-67-tn for more information). A cannula is placed in the trachea for ventilation. Make a longitudinal skin and muscle incision opening the abdomen from the diaphragm to the throat. Cut the diaphragm free from the ribs. Open the thorax following the bone-cartilage border on the left and right sides parallel to the sternum from the diaphragm cranially to the first rib. Turn the complete anterior thoracic wall upwards over the head to expose the heart. Remove the pericardium. Separate the ascending aorta from connective tissue and the pulmonary artery using blunt dissection. Preplace a thread around the aorta.

Prepare for insertion of the aortic cannula: Prime the cannula to remove air bubbles and allow a small stream of perfusate during insertion. Clamp the vena cava above the diaphragm to minimize bleeding. Sprinkle the heart with cold physiological saline (4° C) so the heart slows down and stops beating. Incise the pulmonary artery to avoid distension of the right ventricle. Incise the aorta as far cranially as possible and insert the cannula, taking care that the position of the cannula is not too low to impede the aortic valves or the coronary ostia. Tighten the thread around the end of the cannula. Fully perfuse the heart. Completely isolate and remove the heart for transfer to the Langendorff apparatus.

REFERENCES

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

Yasutake M et al, "Na+/H+ Exchange and Reperfusion Arrhythmias: Protection by Intracoronary Infusion of a Novel Inhibitor," AJP 1994; 267: H2430-40 (452A)

Galiñanes M et al, "Can Ischemic Preconditioning Ensure Optimal Myocardial Protection When Delivery of Cardioplegia Is Impaired?" Circulation 1995; 92 (Pt 2): 389-94. (580A)

Bouma P et al, "Nitric Oxide is An Important Determinant of Coronary Flow in the Isolated Blood Perfused Rat Heart," Basic Res Cardiol 1992; 87: 570-584. (327A)

Bouma P et al, "Coronary Arterial Inflow Impediment during Systole is Little Affected by Capacitive Effects," Am J Physiol 1993; 263: H715-21. (328A)

Zhang J et al, "Endothelin-1 Induces Direct Constriction of Hepatic Sinusoids," AJP 1994; 266(29): G624-G652. (462A)

Habazettl H et al, "Improvement in Functional Recovery of the Isolated Guinea Pig Heart after Hyperkalemic Reperfusion with Adenosine," J Thorac & Cardiovasc Surg, 1996; 111: 74-84. (599A)

Szczesna-Cordary D et al, "Myosin regulatory light chain E22K mutation results in decreased cardiac intracellular calcium and force transients," FASEB J 2007; 21(14): 3974-85.

Sambandam N et al, "Chronic activation of PPAR is detrimental to cardiac recovery after ischemia," AJP Heart Circ. Physiol. 2006; 290: H87-H95.

Skrzypiec-Springa M et al, "Isolated heart perfusion according to Langendorff—Still viable in the new millennium," J Pharmacol Toxicol Methods. 2007; 55(2):113-26.

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