



SI-LANG2

Two column "In-vitro" Langendorff Heart Perfusion System

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INSTRUCTION MANUAL

Serial No. _____

011713

World Precision Instruments

SI-LANG2



CONTENTS

ABOUT THIS MANUAL	1
INTRODUCTION	2
Cautions and Warnings	3
Unpacking	3
INSTRUMENT DESCRIPTION	4
Hardware Description	4
Setup	5
Plumbing the System	12
Temperature Regulation Circuit	14
Carbogen Circuit	16
Perfusion Circuit	17
Filling The System	19
Filling the Temperature Regulation Circuit	19
Filling the Perfusion Circuit	19
Installing the Sensors	20
Pressure Sensors	20
Temperature Sensor	21
Stimulation Electrodes (optional)	22
MAP (ECG) Electrodes (optional)	24
OPERATING INSTRUCTIONS	26
Mounting the Preparation	27
Operating the Valve Taps	28
Constant Pressure Mode	28
Constant Flow Mode	29
MAINTENANCE	30
QUICK REFERENCE CHECKLIST	30
APPENDIX A: TUBING INFORMATION	31
APPENDIX B: EPP-01 PERISTALTIC PUMP	33
General Description	33
Specifications	33
Installation Instructions	33
Tubing Inspection	34
Tubing Replacement	34
INDEX	35
WARRANTY	36
Claims and Returns	36

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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a **CAUTION**. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a **WARNING**. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and **TIPS** contain helpful information.

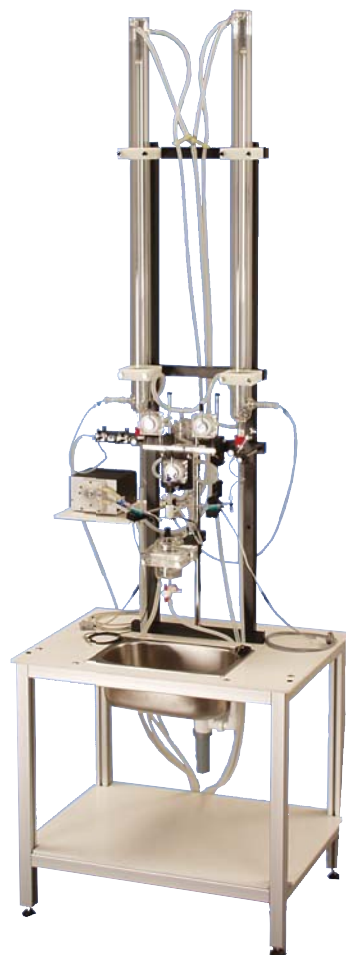


Fig. 1–The two-column Langendorff system is shown. The current model discussed in this manual comes with a stainless steel tray, in place of the sink.

INTRODUCTION

Studies on coronary circulation can easily be accomplished using an apparatus known as a Langendorff system. These devices perfuse isolated heart preparations from small mammalian species through a cannula inserted into the aorta of the heart. The perfusion buffer flows into the heart through the aorta and puts pressure on the aortic valves to close. The perfusion fluid has nowhere to go but through the coronary arteries that come off the side of the aorta. Since the flow of blood into the heart during this type of perfusion is the reverse of normal blood flow, Langendorff methods are known as retrograde perfusions. Also, the hearts subjected to Langendorff perfusion are described as being non-working since the heart does not work as it does *in vivo*. Langendorff systems are useful for measuring the effectiveness of drugs on the vasodilation and vasoconstriction of coronary arteries, and the resultant effects of increased or decreased cardiac perfusion. The second perfusion column permits the heart to be perfused at a different pressure with the rotation of a valve tap.

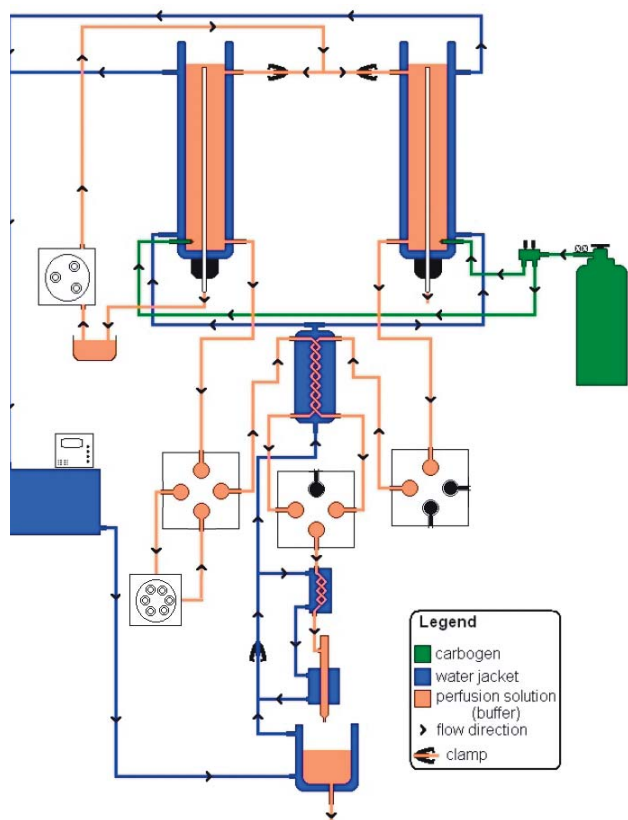


Fig. 2—This plumbing diagram shows how oxygen, water and perfusion buffer run through the Langendorff system.



Cautions and Warnings



CAUTION: Unpacking and installing the system requires more than one person. Beware of the heavy weight of the heavy-duty parts (for example, stands, larger glass or Plexiglas units, etc.).

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 36 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 36 of this manual.

INSTRUMENT DESCRIPTION

Hardware Description

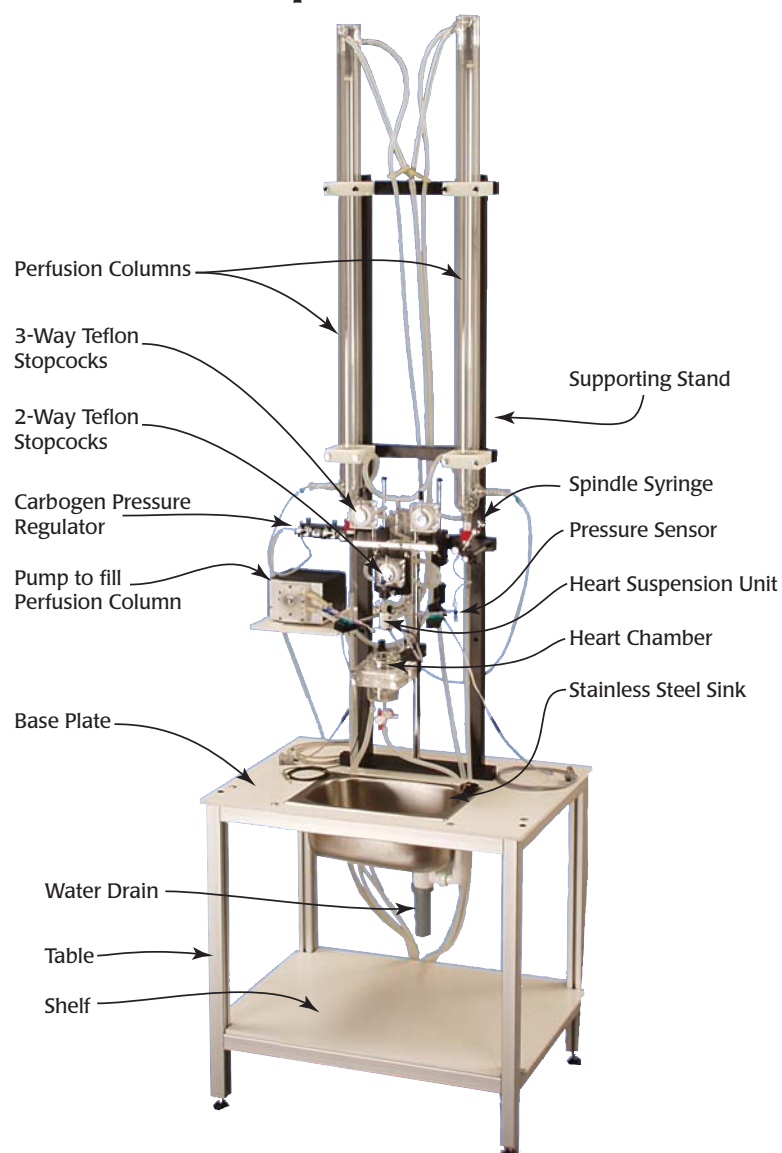


Fig. 3—The components of this Langendorff are labeled.



Setup

1. The table that supports this Langendorff system is delivered in pieces with the legs, cross beams, shelf and table top packed separately. Carefully remove the wrapping from the components. Keep the foam pad in the stainless steel tray on top of the table to prevent damaging the edges of the table. The table top is designed to collect any fluids that might drip from the system. Lay the table top face down on a work table or the floor (Fig. 4).



Fig. 4—Turn the table upside down.

2. Install the table legs.



Fig. 5—(Left) Position the H-shaped legs of the table.

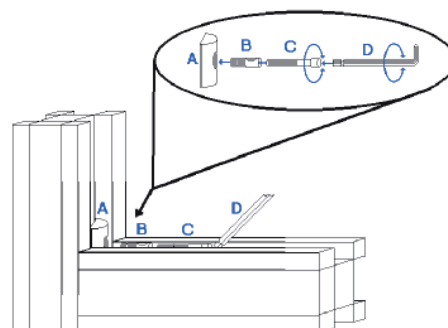


Fig. 6—(Right) Schematic shows how to lock the table legs into place.

- A. Align the top of one of the "H" shaped legs of the table against the cross beams mounted on the bottom of the table (Fig. 5).
- B. Slide the spring-loaded nuts (A) into the tracks of the legs and align them with the threaded tubes (B) in the table frame (Fig. 6).
- C. Push the hex screws (C) into the threaded tubes with the Allen wrench (D) (Fig. 7).
- D. Thread the screws into the nuts and rotate them until they are snug (Fig. 8).
- E. Attach the other leg to the table top in the same manner as the first leg.

SI-LANG2



Fig. 7–(Left) Push the hex screws into place.

Fig. 8–(Right) Tighten the screws.

3. Attach the lower cross beams between the two sets of legs in the same manner as the legs are attached (Fig. 9).



Fig. 9–(Left) Attach the cross bars.

Fig. 10–(Right) Place the shelf under the table.

4. Setup the table.
 - A. Turn the table over and place it where the system will be used. Check the stability of the table. Adjust the height of the feet on the table to level it.
 - B. Place the shelf on the lower crossbars of the table (Fig. 10).
 - C. Remove the foam pad from the tray top of the completed table.
5. Attach the frame rack of the system to the table.
 - A. With the assistance of another person, align the holes on the bottom of the frame rack with the mounting holes on the table top (Fig. 11).

- B. As one person holds the frame rack in position, the other person should use four Allen screws and flat washers to fix the frame rack to the top of the table. Tighten the screws firmly.

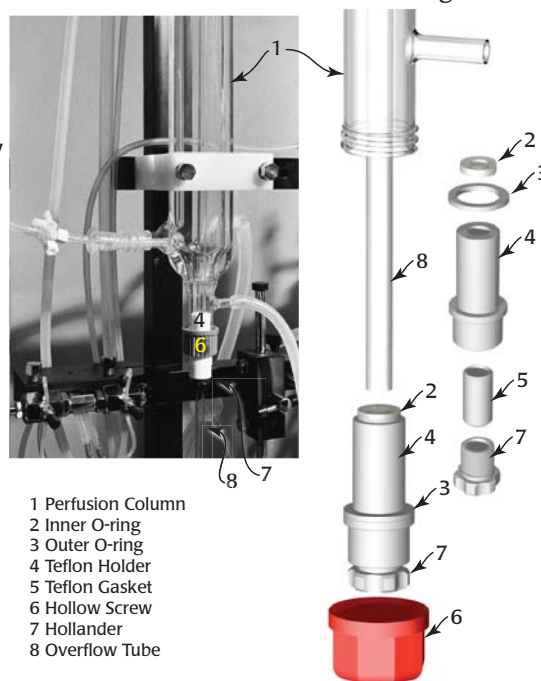


Fig. 11—Attach the frame rack.

NOTE: Assemble the perfusion column and the overflow tube before mounting them on the frame rack.

6. Carefully remove the perfusion columns and the overflow tubes from the packing box. Lay them down on a work table and make sure that they are secure.
- The overflow tube has already been fitted with parts (1-5, 7 in Fig. 12) that are needed for mounting it in the perfusion column, and a silicone grommet near its top to protect the tube if it touches the inside of the column.
 - The perfusion column has already been fitted with the blocks that mount it to the frame rack, and a hollow nut (6 in Fig. 12) on the bottom of the column that is used to secure the overflow tube in the column.

Fig. 12—The exploded view shows how the perfusion column fits together.



7. Assemble the perfusion columns.
- Remove the hollow nut from a column.
 - Carefully feed the upper end of an overflow tube, with its grommet, through the opening in the bottom of a perfusion column until the shoulder of the Teflon tube support (4 in Fig. 12) touches the bottom of the column.
 - Reinstall the hollow nut over the end of the overflow tube and onto the bottom of the perfusion column.
 - Finger-tighten the hollow nut to secure the overflow tube in the column.

CAUTION: Be sure the inner (2) and outer (3) O-rings and the Teflon gasket (5) are installed on the tube support (4) before assembling the perfusion column.

NOTE: The height of the overflow tube sets the pressure used to perfuse the heart in Langendorff mode. To adjust the height of the overflow tube, loosen the knurled nut (7 in Fig. 12) and carefully slide the overflow tube to the correct position. If the tube does not slide, loosen the knurled nut a little more. Once the tube is in position, finger-tighten the knurled nut to secure the overflow tube.

E. Repeat Steps A through D for the second perfusion column.

8. Mount one of the assembled perfusion column to the left side of the frame rack (Fig. 13). The mounting blocks for the perfusion column are already positioned on the column and should align with the mounting holes on the frame rack. With the assistance of another person, attach the perfusion column and its mounting blocks to the frame rack using the four (4) cap screws provided. Mount the second perfusion column to the right side of the frame rack using the same procedure used on the first column.



Fig. 13—Mount the perfusion column to the left side of the frame.

9. Mount the valve tap assembly (Fig. 15) to the central crossbar of the frame rack.

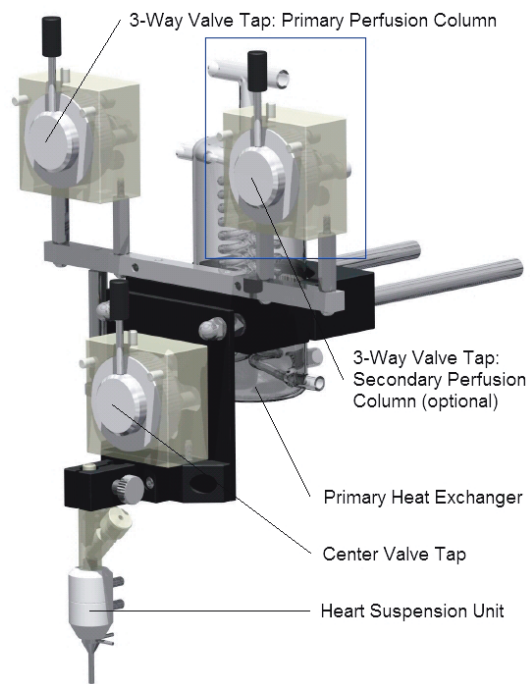


Fig. 14—The labeled diagram shows components of the valve tap assembly.

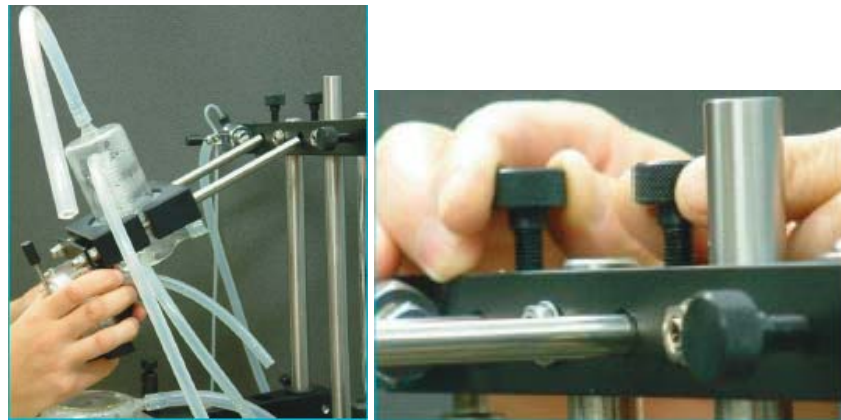


Fig. 15—(Left) Mount the valve tap assembly

Fig. 16—(Right) Secure the rods of the tap valve support to the crossbar.

- A. Loosen the two knobs on the holes in the center of the crossbar (Fig. 16).
- B. Push the rods of the tap valve support into the holes on the crossbar.
- C. Tighten the knobs to secure the tap valve assembly to the frame rack.

10. Mount the carbogen manifold assembly (Fig. 17) to the left end of the central crossbar. The regulators and the tubing are already mounted to the manifold. The manifold is mounted using a single cap screw.



Fig. 17—Mount the carbogen manifold.

11. Mount the shelf for the pump that fills the perfusion columns to the left side of the frame rack (Fig. 18).
 - A. Align the mounting hole of the shelf platform with the hole on the left upright of the frame rack. This hole is about 25cm above the tray top. Secure the platform with the screw and nut provided.
 - B. Place the filling pump that is supplied with system on the shelf



Fig. 18—Mount the pump shelf.

12. Locate the bracket for the heart chamber on vertical stainless steel rod mounted between the middle and lower crossbars of the frame rack.
 - A. Loosen the T-bar screw on the back of the bracket to adjust the position of the bracket.
 - B. Align the bracket so that it faces forward and is high enough for the heart chamber and its drain to safely clear the top of the table.
 - C. Tighten the T-bar screw to lock the bracket in position.
13. Mount the heart chamber and its support to the bracket using the cap screws provided (Fig. 19). Use the upper sets of holes on the bracket and the chamber support.

NOTE: Loosen the T-bar screw to rotate and raise/lower the heart chamber so that it aligns with the heart suspension unit mounted on the tap valve assembly. Likewise, the tap valve assembly and the heart suspension unit can be moved forward and back to align with the heart chamber by loosening the knobs on the middle crossbar.

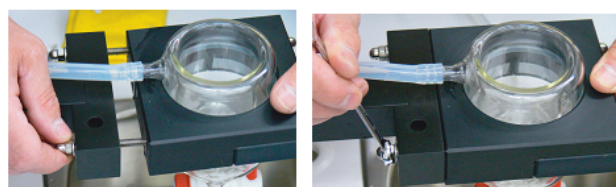
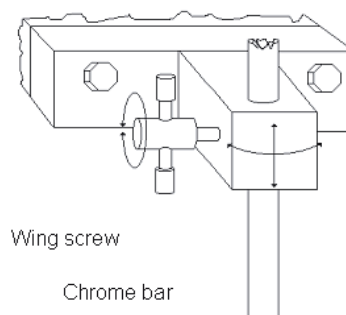


Fig. 19—Mount the heart chamber.



14. Mount the heart suspension unit to the front of the valve tap assembly (Fig. 21). Make sure there is enough clearance between the valve tap assembly and the heart chamber to mount the suspension unit.

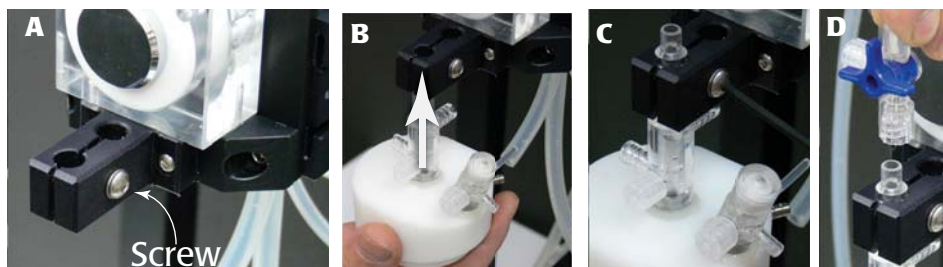


Fig. 20—Follow the steps to mount the heart suspension unit.

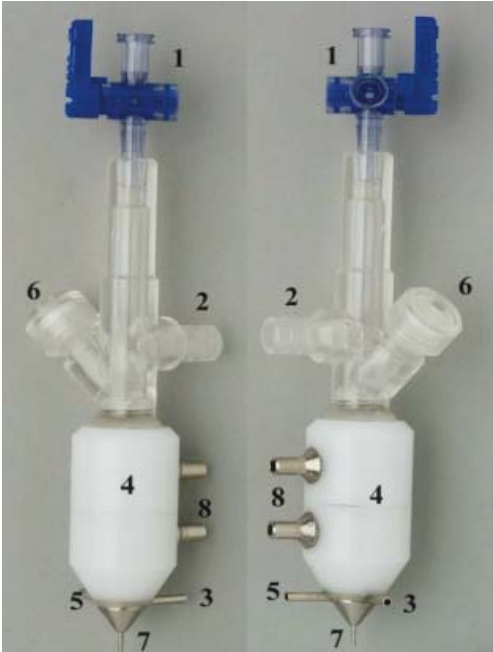
Fig. 21— (Left) The heart suspension unit is mounted.

- A. Loosen the screw on the side of the mounting bracket in the front of the valve tap (Fig. 20, A).
- B. Carefully push the stem of the heart suspension unit into the front mounting hole (Fig. 20, B and C).
- C. Gently tighten the mounting screw to hold the suspension unit snugly in place.



CAUTION: Do not overtighten the mounting screw! Too much pressure on the stem of the suspension unit will cause it to crack.

- D. Attach a 3-way stopcock, with an attached piece of silicone tubing, to the top of the stem for the purpose of flushing and draining the heart suspension unit. (Fig. 20, D)



1	Bubble trap (usually with a 3-way plastic tap on the top)
2	Inflow of the buffer
3	Sensor connection to measure buffer temperature
4	Heating unit to compensate for temperature loss
5	Sensor connection to monitor column pressure
6	Port for drug administration (Silicone membrane for bolus administration or infusion)
7	Cannula for suspending the heart, according to its size
8	Water jacket inlets and outlets

Fig. 22–The heart suspension unit is shown from two different angles.

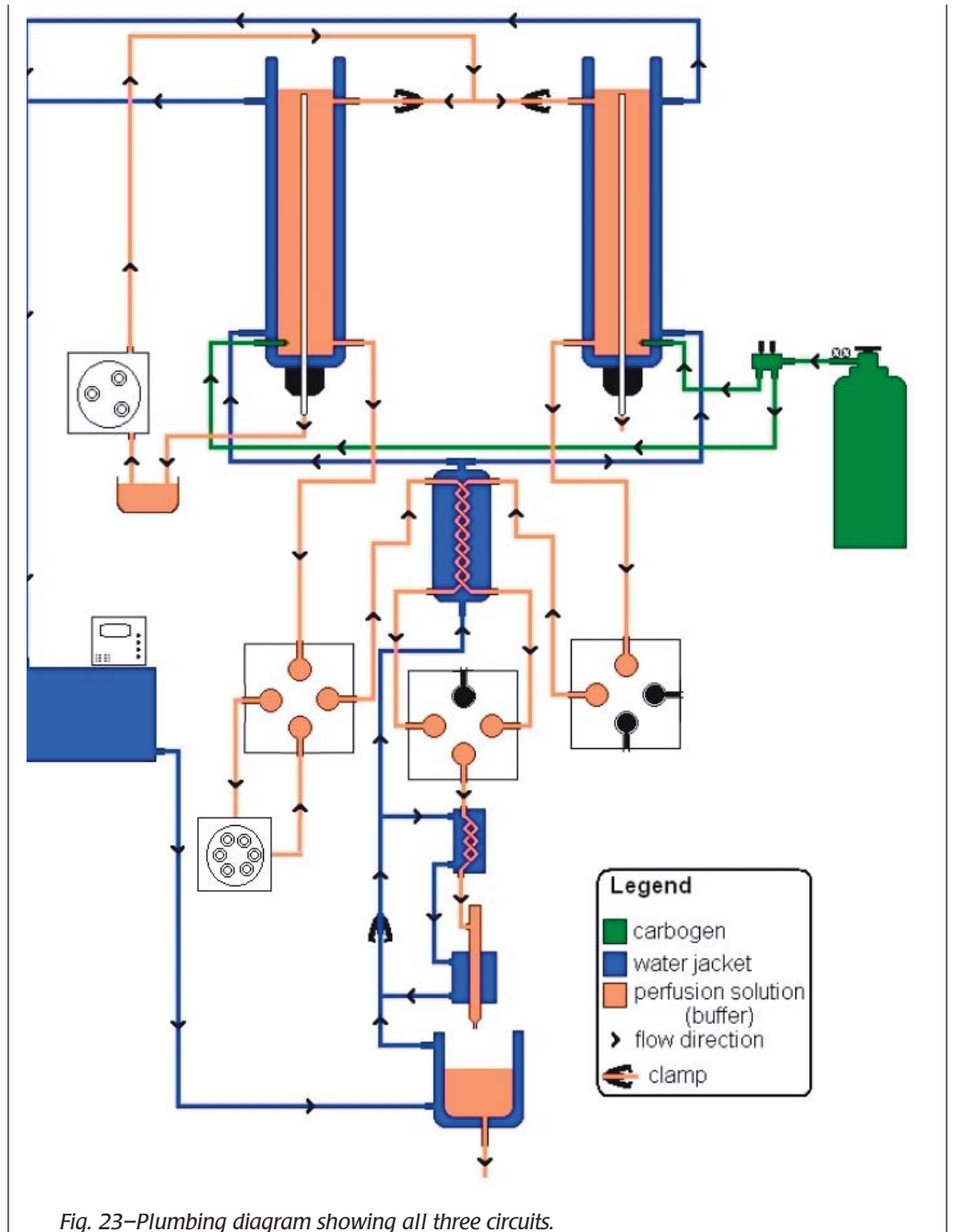
Plumbing the System

The plumbing of Langendorff and working heart systems are organized into three distinct circuits (Fig. 23):

- The regulation of the temperature of the organ and the perfusion buffer
- The movement of perfusion fluid in the system
- The oxygenation of the perfusion fluid and the organ.

The circuits are coded in different colors and the arrows indicate the direction of water, perfusion buffer and oxygen movement.

Diagrams of the individual circuits (Fig. 24, Fig. 26, Fig. 28) that show only the components that are related to each circuit are useful for completing the tubing connections. Many of the components in a circuit may already have tubing attached to them. In some cases, completing sections of the circuit are as simple as connecting the open end of the tubing on one component to another component. A table that lists the lengths and diameters of the tubing pieces in all three circuits, and a numbered diagram that pinpoints the location of each piece of tubing in each circuit, can be found at the end of this manual.



Temperature Regulation Circuit

Complete the connections between the water jackets of the required components and the recirculating water bath using the following directions (Fig. 24).

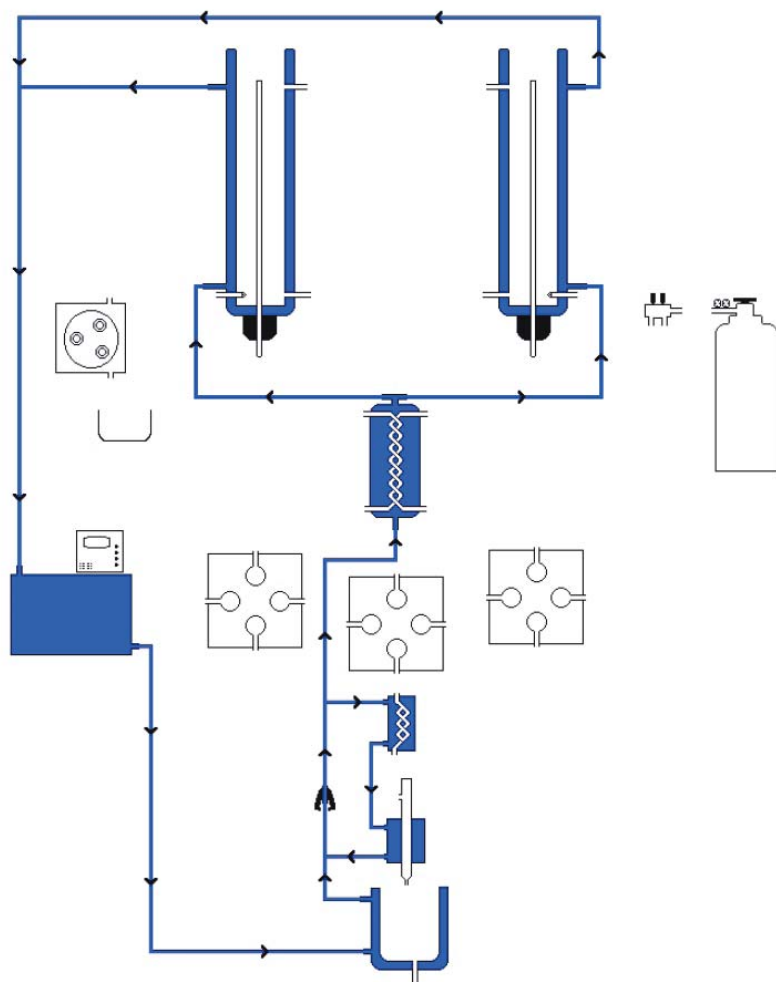


Fig. 24–Water Jacket plumbing diagram.

1. Connect the outlet of the recirculating water bath to the water jacket inlet at the bottom of the heart chamber.



2. Connect the water jacket outlet at the top of the heart chamber to the large inlet on the lower fork of the water bypass assembly.
3. Connect the small tube on the lower fork of the water bypass assembly to the lower water jacket inlet on the back of the heart suspension unit.
4. Connect the upper water jacket outlet of the heart suspending unit to the water jacket inlet on the front of the secondary heat exchanger using a short silicone tube.
5. Connect the small tube on the upper fork of the water bypass assembly to the water jacket outlet on the back of the secondary heat exchanger.



Fig. 25–Heart suspension unit and secondary heat exchanger showing the tubing connections between these components.

6. Connect the large outlet of the upper fork of the water bypass assembly to the water jacket inlet on the bottom of the primary heat exchanger using a piece of silicone tubing.
7. On the top of the primary heat exchanger, connect the water jacket outlet that points to the left to the water jacket inlet on the lower side of the left perfusion column with a piece of silicone tubing. Likewise, connect the water jacket outlet of the primary heat exchanger that points to the right to the water jacket inlet on the lower side of the right perfusion column.
8. Attach a piece of silicone to the water jacket outlet on the upper side of each perfusion column. Using a T-connector, attach the tubes on the water jacket outlets of each perfusion column to a tube attached to the inlet of the recirculating water.



CAUTION: When the circuit is complete, make sure it is filled with deionized water only.

Carbogen Circuit

Complete the connections between the carbogen supply and the aeration frits in the reservoirs using the following directions (Fig. 26).

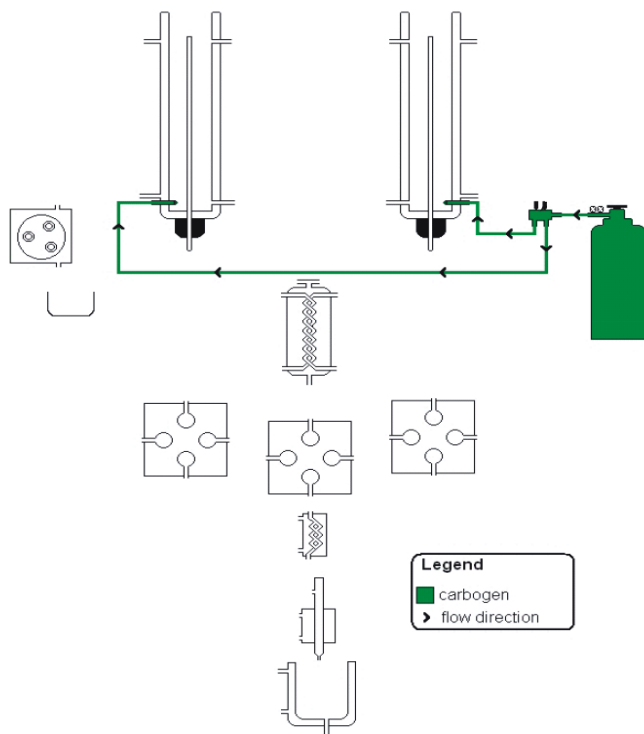


Fig. 26—Carbogen circuit plumbing diagram.

1. Connect the pressure regulator and valves on the carbogen cylinder to the manifold and regulators on the left side of the frame rack using the thin silicone tubing already attached to the inlet of the carbogen manifold.
2. A piece of silicone tubing has been attached to the outlet of each carbogen regulator of the aeration manifold that is located on the left side of the frame rack. Connect the regulator with the shorter piece of silicone tubing to the Luer fitting and 3-way stopcock on the aeration frit of the left perfusion column (Fig. 27).
3. Connect the regulator with the longer piece of tubing to the Luer fitting and 3-way stopcock of the aeration frit of the right perfusion column.



Fig. 27—(Right) Carbogen connection.

Perfusion Circuit

Complete the connections between the components that perfuse the heart using the following directions (Fig. 28).

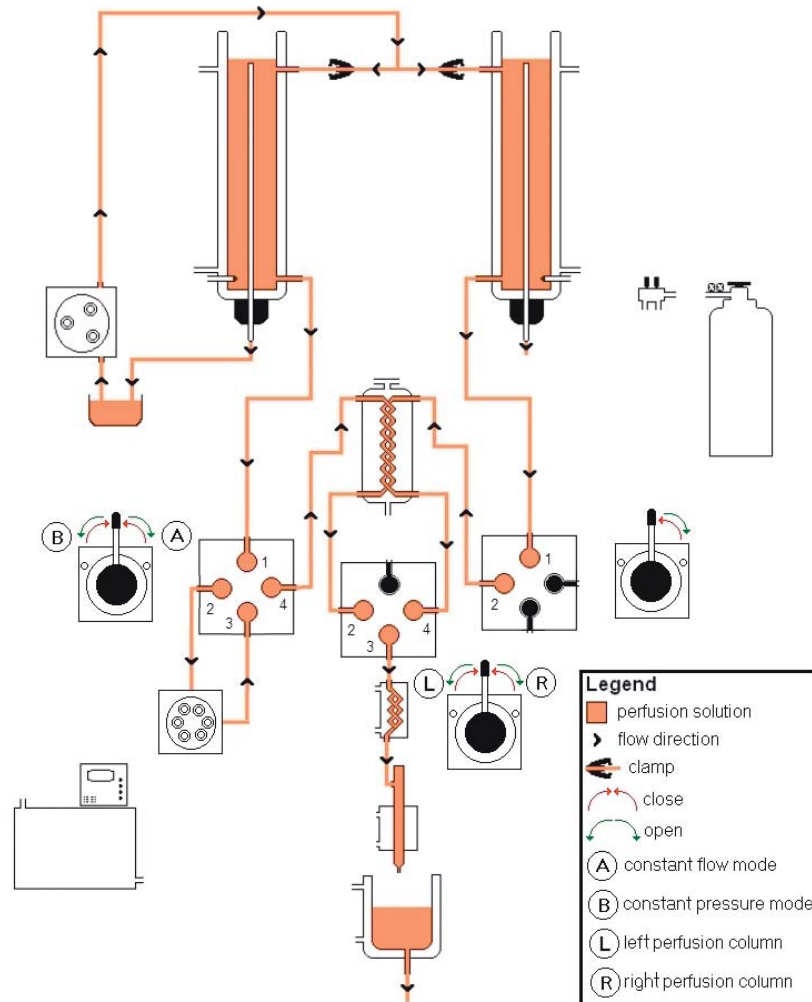


Fig. 28—Perfusion circuit diagram.

1. Place a carboy, which will eventually hold fresh perfusion buffer, on the left side of the shelf under the tray top.
2. Place the open end of the silicone tubing, which is attached to the bottom of the overflow tube in the perfusion column, through the hole on the left side of the tray top and into the carboy that will hold perfusion buffer. Make sure the end of this tubing does not go below the surface of the buffer when the carboy is filled.

3. Connect a piece of silicone tubing to the inlet tube of the pump used to fill the perfusion columns. Put the open end of this tubing into the buffer carboy. The tubing should be long enough to reach the bottom of the carboy.
4. Attach another piece of silicone tubing to the outlet of the fill pump. Connect the other end of this tubing to the port in the center of a plastic T-connector.
5. Connect the port on the left side of the T-connector to the buffer inlet at the top of the left perfusion column using a short silicone tube equipped with a pinch valve. Do the same for the port on the right side of the T-connector and the right perfusion column.
6. Use a piece of silicone tubing to connect the buffer outlet at the bottom of left perfusion column to the upper inlet port (stub 1) on the back of the left 3-way valve.
7. Use another piece of silicone tubing to connect the left outlet (stub 2) of the left 3-way valve to the inlet of the precision peristaltic pump.
8. Connect the outlet of the peristaltic pump to the bottom inlet (stub 3) of the left 3-way valve with another piece of silicone tubing.
9. Connect the right outlet (stub 4) of the left 3-way valve to the upper left inlet of the primary heat exchanger with a piece of silicone tubing.
10. Use a piece of silicone tubing to connect the lower left outlet of the primary heat exchanger to the left inlet (stub 2) of the central valve.
11. Use another piece of silicone tubing to connect the bottom outlet (stub 3) of central valve to the back inlet of the secondary heat exchanger (Fig. 25).
12. Connect the front outlet of the secondary heat exchanger to the upper buffer inlet on the back of the heart suspension unit with a short piece of silicone tubing (Fig. 25).
13. Use a piece of silicone tubing to connect the buffer outlet at the bottom of the right perfusion column to the upper inlet port (stub 1) on the back of the right 3-way valve.
14. Connect the left outlet (stub 2) of the right 3-way valve to the upper right inlet of the primary heat exchanger with a piece of silicone tubing.
15. Use a piece of silicone tubing to connect the lower right outlet of the primary heat exchanger to the right inlet (stub 4) of the central valve.
16. Connect a piece of silicone tubing to the drain on the bottom of the heart chamber. Place the open end of this tubing through the hole on the right side of the tray top and into a second carboy that is designated as the waste container for the fluids used in the system. Make sure the end of this tubing does not go below the surface of the buffer when the carboy is filled.



Filling The System

Filling the Temperature Regulation Circuit

1. Follow the directions for operating the circulating heated water bath that are provided by its manufacturer.
2. Fill the reservoir of the circulating water bath with deionized water.
3. Turn on the water bath and set the temperature needed for the experiment.
4. Activate the pump of the bath to move water from the reservoir into the water jackets of the columns and chambers. As water moves into the system, replenish the water in the reservoir until the water jackets are full.



CAUTION: Most recirculating water baths have a safety sensor that will turn off the heater and pump automatically if the water level drops below a critical level. If the water bath turns off because the water level is low, add water to the reservoir and restart the water bath.

5. Check the water level in the reservoir every day. Add water to the reservoir, if needed.

Filling the Perfusion Circuit

1. Make at least 5L of perfusion buffer (Krebs-Henseleit or similar). This is the volume required to fill the perfusion circuit of the system. Fill the clean carboy on the shelf under the left side of the tray top with the fresh buffer.
2. Place the silicone tubing on the inlet of the fill pump under the surface of the buffer in the carboy. Place the tubing on the overflow of the perfusion column in the carboy, but not under the surface of the buffer.
3. Set the desired hydrostatic pressure used to perfuse the heart by moving the overflow tube of each perfusion column up or down. Determine the height that the open end of the overflow tube needs to be above the aorta of the heart to set the desired perfusion pressure.

Calculate the height needed by multiplying the desired perfusion pressure (in mmHg) by the 13.6 conversion factor. For example, to achieve a perfusion pressure of 60mmHg, the end of the overflow tube needs to be 816mm above the level of the aorta of the isolated heart.

NOTE: To adjust the height of the overflow tube, loosen the knurled nut (7 in Fig. 12) and carefully slide the overflow tube to the correct position. If the tube does not slide, loosen the knurled nut a little more. Once the tube is in position, finger-tighten the knurled nut to secure the overflow tube.

4. Start the flow of carbogen to the aeration inlet of the perfusion column. Open the valves on the cylinder of carbogen. Open the regulators on the aeration manifold and the 3-way stopcocks on the aeration inlet of the column. Begin the flow of carbogen through the aeration line before the perfusion fluid is added to the system

5. Turn on the fill pump to move buffer into the perfusion columns. The columns will continue to fill until the fluid level reaches the top of a overflow tube. Any excess fluid in a perfusion column will flow down its overflow tube and back into the fresh buffer carboy. Keep the pump running while the rest of the system is being filled.
6. Rotate the tap on the left 3-way valve to the constant flow position (Position A on Fig. 28).
7. Turn on the precision peristaltic pump to move perfusion buffer through the pump and the left 3-way valve to the primary heat exchanger.
8. Open the central valve by rotating the tap to the left, and then to the right, to fill the remaining components in the perfusion system with buffer. When the perfusion system is filled properly, buffer should flow out of the end of the aortic cannula without bubbles.
9. When the buffer is bubble-free close the central valve and 3-way valves and turn off the precision peristaltic pump. Keep the column filling pump running.
10. Turn on the recirculating water bath and begin warming the system and perfusion buffer. Start warming the system 30 to 60 minutes before starting the isolation of the heart. Also, it takes at least 30 minutes for the P_{O_2} and P_{CO_2} values of the buffer to reach equilibrium.

Installing the Sensors

Pressure Sensors

1. The bodies of the pressure transducers are installed in holders on the left and right sides of the 2-way valve bracket (Fig. 29). Push in the spring loaded plate in the center of the holder and slide the sides of the sensor body under the retaining rails of the holder. Make sure the sensor is oriented so that the cable of sensor is pointed to the rear of the system.

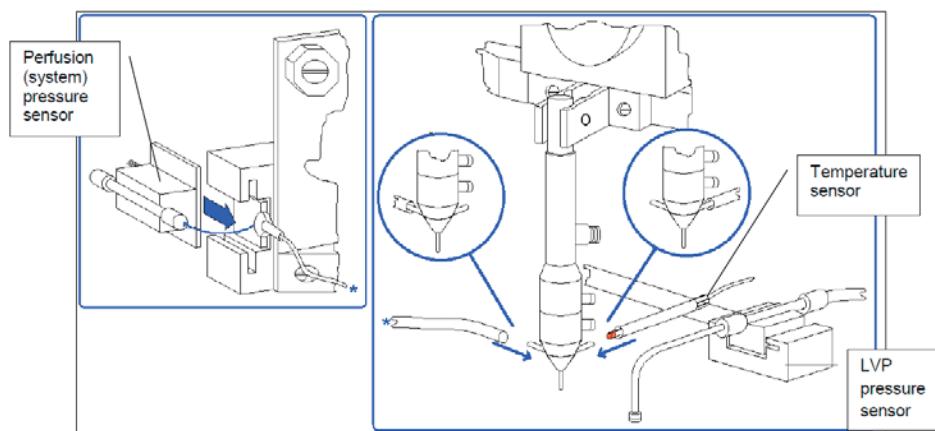


Fig. 29—Install the pressure transducers in the holders on the left and right sides of the valve tap bracket.



2. Install the sensor that measures the perfusion pressure in Langendorff mode on the left side of the valve tap assembly.
 - A. Attach a 3-way stopcock with an empty 2mL syringe to the rear port of the pressure sensor to pull buffer into the sensor and its PVC line, and to close off the syringe when measurements are made.
 - B. Attach tubing, which matches the pressure port on the heart suspension unit, to the front of the pressure sensor
 - C. Attach the open end of the tubing to the lower port on the back of the heart suspension unit.
3. If the left ventricular pressure (LVP) is going to be measured, install another sensor on the right side of the valve tap assembly.
 - A. Attach a PVC cannula and needle to the front of the LVP sensor. The cannula should match the balloon being used as the catheter in the left ventricle of the heart being studied.
 - B. Attach a 3-way stopcock to the rear port of the LVP sensor.
 - C. Attach the tubing from the spindle syringe, which is used to set the diastolic pressure of the heart, to the stopcock on the rear of the LVP sensor.

Temperature Sensor

1. Connect the plastic tube around the end of the temperature sensor to the stainless steel tube on the side of the heart suspension unit. The end of the temperature sensor should not touch the metal tube on the inlet.
2. Mount the cable clamp for the lead wire of the temperature sensor on the back of the heart chamber support bracket while the heart chamber is lowered.
3. With the heart chamber still lowered, place the cable of the temperature sensor in the slot of the clamp (Fig. 30). Gently tighten the retaining screw to hold the cable in place. Make sure the cable is aligned so that it does not touch the heart, or it is not damaged when the heart chamber is raised.



Fig. 30—(Left) Clamp for cable of the temperature sensor.



Fig. 31—(Right) The cable from the temperature sensor slides through the slot on the clamp.

Stimulation Electrodes (optional)

1. Screw the mounting post to the bottom of the valve tap assembly (Fig. 32).

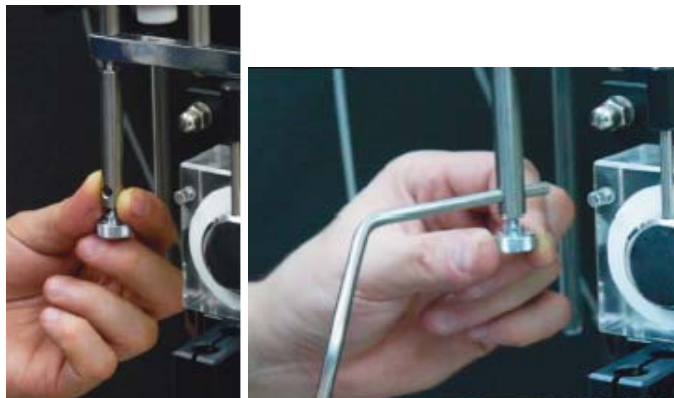


Fig. 32—(Left) Insert the mounting post.

Fig. 33—(Right) Attach the offset arm.

2. Attach the offset arm that suspends the stimulation electrode from the mounting post (Fig. 33).
3. Attach the ball joint bracket of either the monopolar or bipolar stimulation electrode to the end of the offset arm.

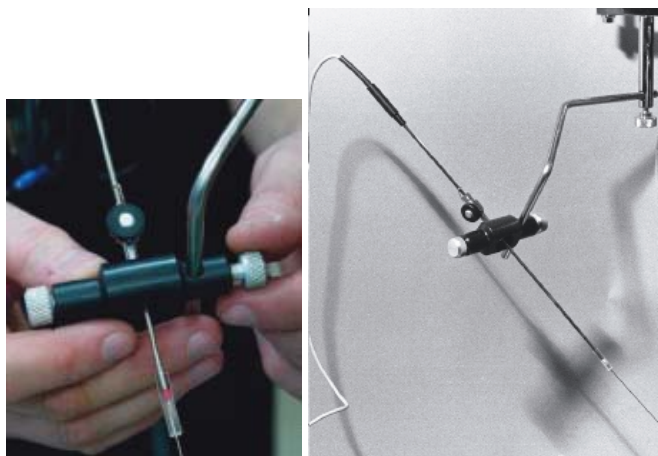


Fig. 34—(Left) Mount the stimulation electrode to the offset arm.

Fig. 35—(Right) The offset arm suspends the stimulation electrode.

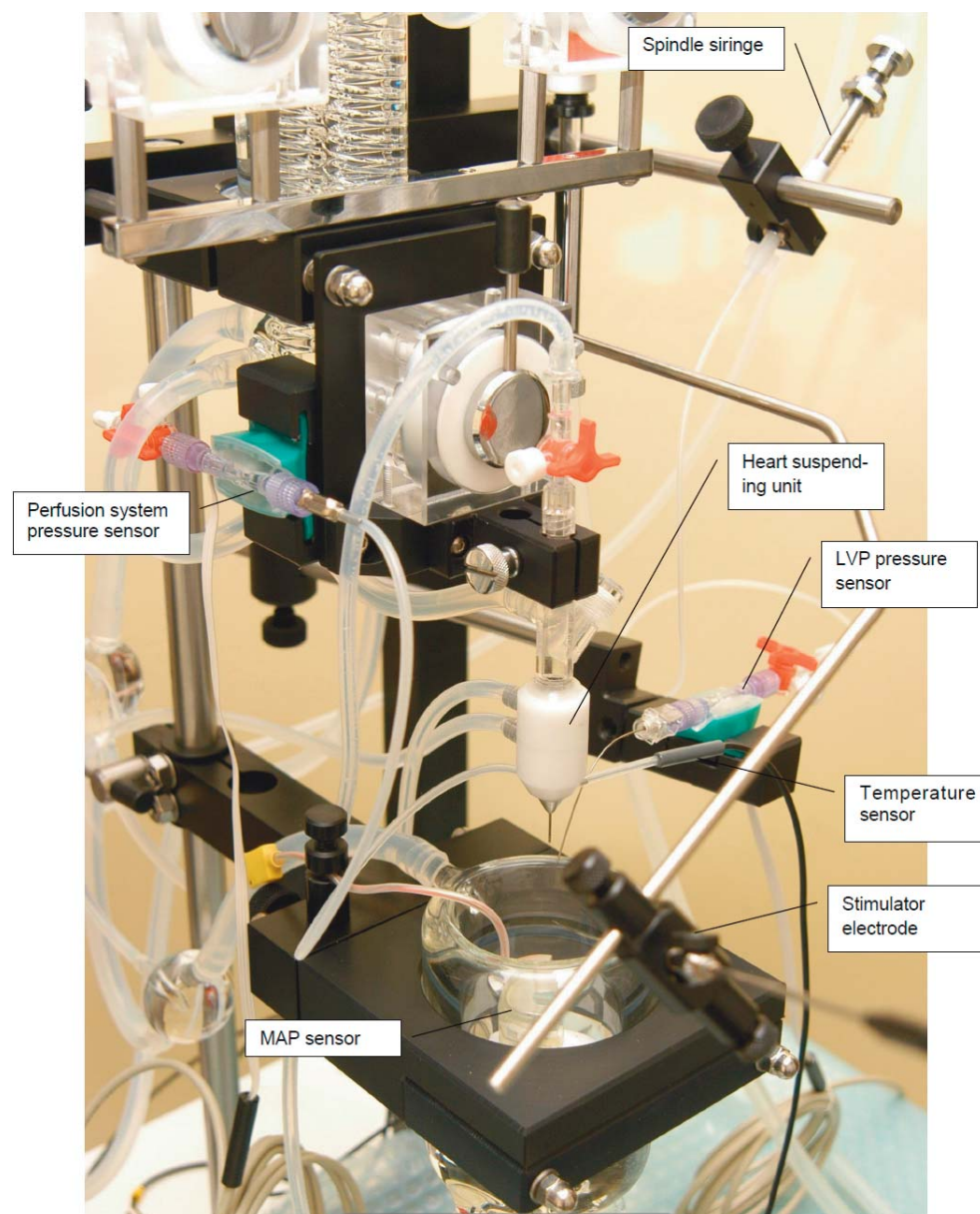


Fig. 36–The complete cannula system is shown.

MAP (ECG) Electrodes (optional)

The MAP sensor is an optional sensor designed to record monopolar action potentials (MAP) from as many as 12 different locations around the heart (Fig. 37) while the heart is being perfused during Langendorff mode.



Fig. 37—(Left) The MAP sensor is an option.

Fig. 38—(Center) Place the MAP sensor inside the heart chamber.

Fig. 39—(Right) Position the heart so that it doesn't touch any of the electrodes on the sensor.

1. To use the MAP sensor, place it in the heart chamber that is filled with perfusion buffer (Fig. 38). Raise the heart chamber and sensor around the heart mounted on the aortic cannula. Make sure the heart does not touch any of the 12 electrodes on the sensor or the sides of the sensor (Fig. 39).
2. Connect the lead of the MAP sensor to the extension cable (Fig. 40). Connect the Canon (D-sub) connector on the end of the extension cable to the input of the fourth channel on the amplifier provided with the system. This amplifier is capable of recording signals from three different positions around the heart at the same time.

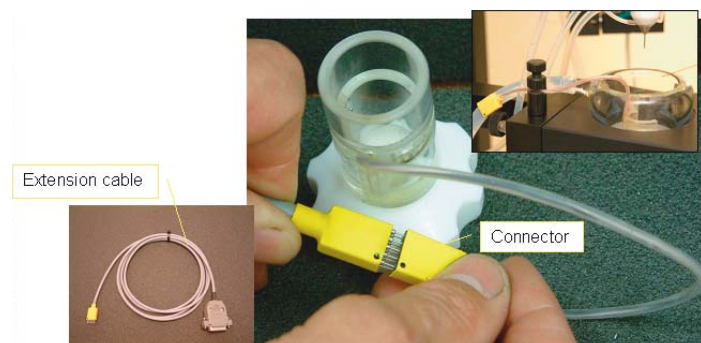


Fig. 40—Connect the MAP sensor to the extension cable.

3. Raise or lower the sensor to adjust the height of the sensor for better alignment of the heart and the electrodes (Fig. 41). Screw or unscrew the base from the sensor to change the height of the sensor as much as 7mm.

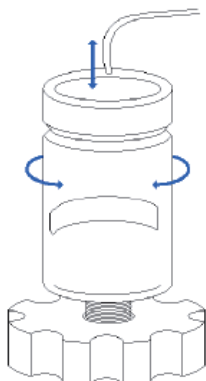


Fig. 41—Rotate the sensor to adjust its height.

4. If different sized hearts are going to be used on this system, or if the electrode array in the sensor is damaged, the sensor can be easily replaced by unscrewing the sensor from the base and attaching a new one (Fig. 42).

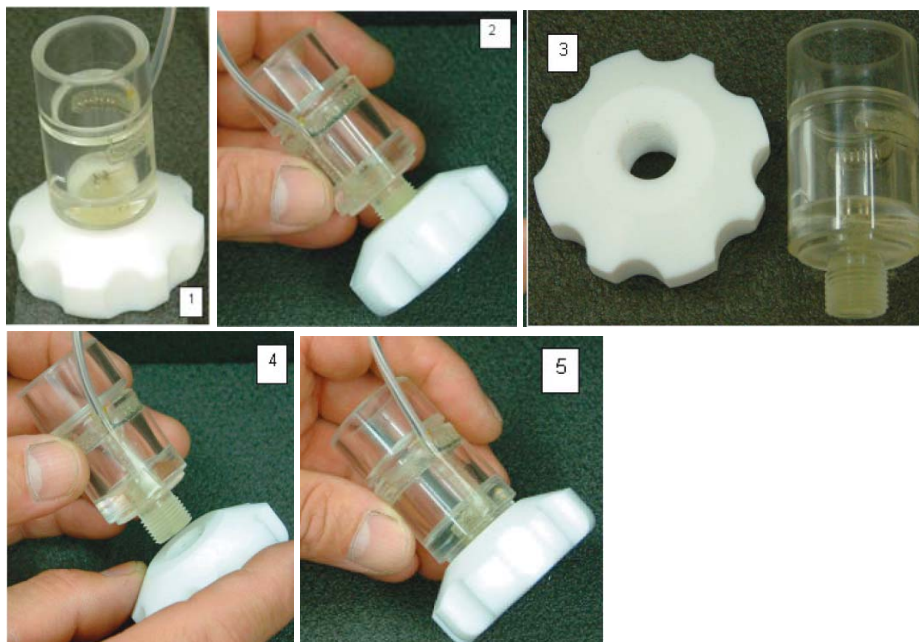


Fig. 42—Steps 1-5 show how to replace the electrode array of the sensor.

OPERATING INSTRUCTIONS

NOTE: A manual that describes the isolation, mounting and perfusion of a heart in a Langendorff or system is available for investigators who are using one of the heart perfusion systems from WPI. Please contact technical support at technicalsupport@wpiinc.com or 866.606.1974 if you need this manual.

For information on the preparation and principles of the Langendorff preparation, refer to the following references:

- Brown, J. M., Terada, L. S., Grosso, M. A., Whitman, G. J., Velasco, S. E., Patt, A., Harken, A. H., Repine, J. E.: *Xanthine oxidase produces hydrogen peroxide which contributes to reop-erfusion injury of ischemic isolated perfused hearts*. J. Clin. In-vest. 1988. 137. 396.
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- Neely, J. R., Grotyohann, L. W.: *Role of glycolytic products in damage to ischaemic myocardium. Dissopciation of adenosine triphosphate leves and recovery of function of reperused ischemic hearts*. Circ. Res. 1984. 55. 816-824.
- Opie, L. H.: *Adequacy of oxygenation of isolated perfused rat heart*. Basc Res. Cardiol. 1984. 79. 300-308.
- Rosenkranz, E. R., Okamoto, F., Buckberg, G. D., Vinten- Johanes, J., Allen, B. S., Laef, J., Bugyi, H., Young, H., Barnard, R. J.: *Studies of controlled reperfusion after ischenia. II. Bio-chemical studies: Failure of tissue adenosine triphosphate leves to predict recovery of contractile function after controlled reperfusion*. J. Thorac. cardiovasc. Surg. 1986 .92. 488-501.
- Scott, M. D., Eaton, J. W., Kuypers, F. A., Chiu, D. T.-Y., Lubin, B. H.: *Enhancement of erythrocyte superoxide dismutase activity: Effect on cellular oxidative defense*. Blood 1989. 74. 2542- 2549.
- Toth, K. M., Clifford, D. P., White, C. W., Repine, J. E.: *Intact human erythrocytes precent hydrogen peroxide mediated dam-age to isolated perfused rat lung and cultured bovine pulmonary artery enbothelial cells*. J. Clin. Invest. 1984. 74. 292-295.



Mounting the Preparation

Helpful hints:

1. Before the heart is isolated, turn the valve tap to a position that allows a slow dripping of the buffer from aortic cannula.
2. Once the heart is removed from the ice cold perfusion buffer, mount it on the aortic cannula as soon as possible. No more than 30 seconds should expire between the time the heart leaves the ice cold buffer and is firmly attached to the cannula and being gently perfused with buffer.
3. Once the isolated heart is fed onto the aortic cannula of the heart suspension unit, tighten the ligature that secures the aorta to the cannula. Tie a second ligature to firmly attach the heart.
4. Once the heart is secured on the cannula, open the valve tap all the way to fully perfuse the heart.
5. If required, rotate the heart around the cannula to place the heart in a suitable position with the left ventricle on the left side suspension unit and the left atrium on the right side of the unit.
6. While the heart is being fully perfused in Langendorff mode, cannulate the left atria with the atrial cannula. To measure the left ventricular pressure, the atrial cannula needs to be inside the left ventricle, not just the pulmonary vein or the left atria. Once the heart is properly positioned, tie off any leaks around the atrial cannula with ligatures.



CAUTION: If the flow from the coronary arteries is low. Check the position of the cannula in the aorta. If the aortic cannula is too far into the aorta, the cannula blocks the entrances (ostia) of the coronary arteries and the flow of perfusion fluid into the coronary arteries and the cardiac musculature is reduced. If the reduction in coronary flow is significant, the cardiac muscles could suffer from hypoxia and start to die.

Operating the Valve Taps

The Langendorff system can be operated in either constant pressure or constant flow mode.

Constant Pressure Mode

In constant pressure mode, the perfusion fluid is driven from the outlet of the perfusion column to the aortic cannula of the heart suspending unit by the pressure created by the height of the perfusion fluid in the column. Along the way, fluid passes sequentially through:

- The left or right 3-way valve tap, which is set to constant pressure mode.
- The primary heat exchanger for heating the perfusion buffer after it leaves the perfusion column.
- The central valve tap, which conducts the perfusion buffer to the aortic cannula or stops it.
- The secondary heat exchanger for reheating the perfusion buffer just before it enters the aortic cannula and the heart.

To operate the Langendorff system in constant pressure mode from the left perfusion column:

- Rotate the tap of the left 3-way valve to position B (Fig. 28 and Fig. 43) to permit the fluid to flow from the outlet of the left perfusion column into this valve through stub 1. When the buffer is flowing, it leaves this valve through stub 4 and goes into the left buffer inlet of the primary heat exchanger.
- Make sure the tap of the right 3-valve tap is rotated to the center (closed) position.
- Rotate the tap of the central valve to the left position (Fig. 28) to permit the flow of the perfusion fluid to the heart suspension unit.

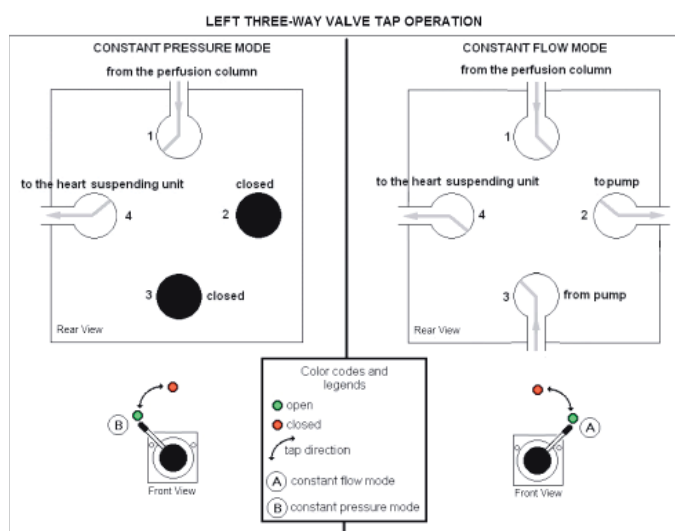


Fig. 43—Valve tap operation for left 3-way valve.



To operate the Langendorff system in constant pressure from the right perfusion column:

1. Rotate the tap of the right 3-way valve to the open position (Fig. 28) to permit the perfusion fluid to flow from the outlet of the right perfusion column into this valve through stub 1. When buffer is flowing, it leaves this valve through stub 2 and goes into the right buffer inlet of the primary heat exchanger.
2. Make sure the tap of the left 3-valve tap is rotated to the center (closed) position.
3. Rotate the tap of the central valve to the right position (Fig. 28) to permit the perfusion fluid to flow from the right outlet of the primary heat exchanger into this valve through stub 4. When the buffer is flowing, it leaves this valve through stub 3 and goes into the inlet of the secondary heat exchanger. Buffer leaving the secondary heat exchanger, it enters the heart suspending unit and exits the aortic cannula.
4. Rotate the tap on either the central or right 3-way valve to the center position to stop the flow of the perfusion fluid to the heart suspension unit.

Constant Flow Mode

In constant flow mode, the perfusion fluid is driven from the outlet of the perfusion column to the aortic cannula of the heart suspending unit by the precision peristaltic pump set to the desired flow rate. Along the way, the fluid passes sequentially through:

- A. The left 3-way valve tap, which is positioned to conduct the perfusion buffer to the heart in constant flow mode (Position A in Fig. 43).
- B. The primary heat exchanger for heating the perfusion buffer after it leaves the perfusion column.
- C. The central valve tap, which conducts the perfusion buffer to the aortic cannula or stops it.
- D. The secondary heat exchanger for reheating the perfusion buffer just before it enters the aortic cannula and the heart.

To operate the Langendorff system in constant flow mode using the pump attached to the left perfusion circuit:

1. Rotate the tap on the left 3-way valve to position A (Fig. 28 and Fig. 43) to permit the perfusion fluid to flow from the outlet of the left perfusion column into this valve through stub 1. When the buffer is flowing, it leaves this valve through stub 2 and goes into the inlet of the precision peristaltic pump. The pump returns the buffer to this valve through stub 3 at a flow rate determined by the speed of the pump. The buffer leaves the valve through stub 4 and enters the primary heat exchanger through its left inlet.
2. Make sure the tap of the right 3-valve tap is rotated to the center (closed) position.
3. Rotate the tap of the central valve tap to the left position (Fig. 28) to permit the perfusion fluid to flow from the outlet of the primary heat exchanger into this valve through stub 2. When the buffer is flowing, it leaves the valve through stub 3 and goes into the inlet of the secondary heat exchanger. Buffer leaving the secondary heat exchanger enters the heart suspending unit and exits the aortic cannula at a flow rate established by the speed of the precision peristaltic pump.
4. Rotate the tap on either the central or the left 3-way valve to the center position to stop the flow of the perfusion fluid to the heart suspension unit.

MAINTENANCE

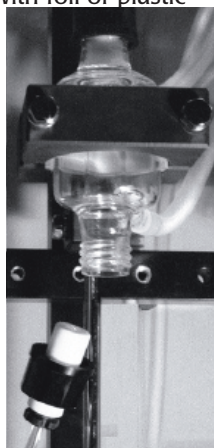
The system should be carefully cleaned every day when the experiment is finished. When used regularly, it is sufficient to rinse the perfusion circuit with deionized water 2 or 3 times, and then empty it. Cover the opening of the perfusion column with foil or plastic wrap to keep dust out of the system.

Buffer solution, accumulating under the outlet stub of the perfusion (Langendorff) column, should be drained off by loosening the hollow nut (6 in Fig. 12) of the overflow tube. The inside of the elastic and atrial chambers can be drained or cleaned by loosening the nut and removing the plug at the bottom of each chamber (Fig. 44).

Fig. 44--(Right) Loosen the nut to drain the elastic or atrial chamber.

If there are deposits, like proteins, in the perfusion circuit, fill the circuit with a dilute (2%) solution of hydrogen peroxide. Leave the solution in the circuit for several hours. Then, rinse with 4–5 changes of deionized water.

Change the deionized water in the recirculating water bath every two weeks. It is advisable to put a small amount of germicide, like 0.01% ROCCAL, in the water.



QUICK REFERENCE CHECKLIST

Carefully read the directions in "Setup" on page 5, "Plumbing the System" on page 12, "Filling The System" on page 19, and "Installing the Sensors" on page 20 before using the following quick reference list.

1. Check if all tubes are properly connected.
2. Check the position of all taps and cocks.
3. Check if all pumps and measuring devices are properly attached to the system.
4. Check if electric current supply is proper and switched on.
5. Fill up the perfusion reservoir with approximately 5L of solution. Start the filling pump and fill up the perfusion buffer carboy.
6. Fill up the recirculating water bath with distilled water and set the proper temperature.
7. Start the recirculating water bath to warm up the water jacket.
8. Start bubbling the perfusion fluid with carbogen gas. The intensity of the bubbling should be adjusted by the regulators to be even and continuous.
9. Remove air bubbles from the perfusion system.
10. Switch on all control and recording devices.
11. Check the calibration of the sensors being used.
12. Check the speed of the precision pump.
13. Prepare the heart.
14. Attach the prepared heart to the system.
15. Start the perfusion of the isolated heart by turning the tap valve to the Open position.



APPENDIX A: TUBING INFORMATION

Mark	Name	Mark	Name
A	Left perfusion column	I	Carbogen tank
B	Right perfusion column	J	Carboy with perfusion fluid
C	Primary heat exchanger	K	Circulating water bath
D	Left 3-way Teflon valve tap	L	Constant flow pump
E	Right 3-way Teflon valve tap	M	Aeration regulator
F	Central Teflon valve tap	N	Perfusion column pump
G	Heart suspending unit	O	Circulating water diverter
H	Heart chamber	P	Secondary heat exchanger

No. of tubing	OD/ID mm	Length mm	No. of tubing	OD/ID mm	Length mm
1	12/8	2500	15	10/6	1500
2	12/8	250	16	10/6	110
3a	6/3	350	17	10/6	110
3b	6/3	50	18	10/6	1500
4	12/8	200	19	10/6	1500
5	6/3	350	20	10/6	140
6	12/8	250	21	10/6	140
7	12/8	310	22	10/6	180
8	12/8	310	23	10/6	180
9	12/8	310	24	10/6	230
10	12/8	120	25	10/6	230
11	12/8	2700	26a	10/6	100
12a	6/3	260	26b	10/6	100
12b	6/3	1160	27	10/6	1200
13	10/6	1500	28a	10/6	1640
14	10/6	1500	28b	10/6	1640

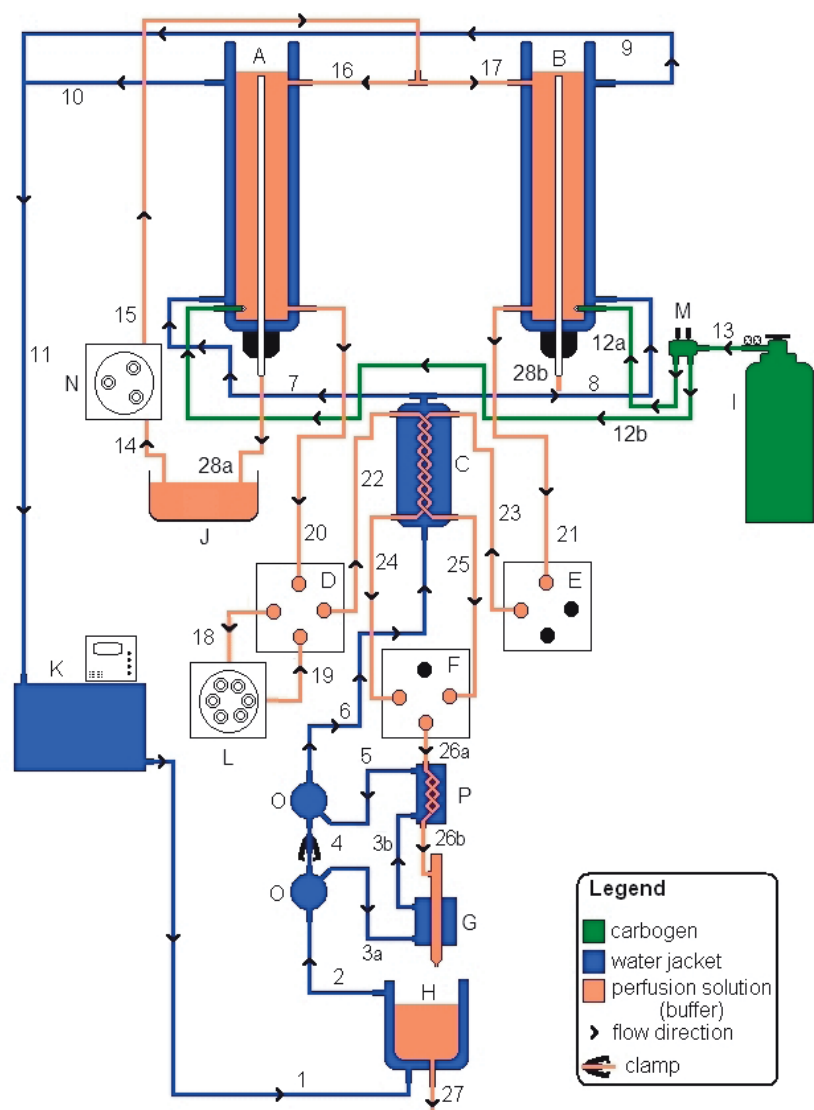


Fig. 45–The numbers on this drawing correspond with the table on the previous page.



APPENDIX B: EPP-01 PERISTALTIC PUMP

General Description

This peristaltic pump (Fig. 46) is used to fill the perfusion column during Langendorff mode. The pump is self-priming and is capable of moving mediums with a wide variety of viscosities from air to heavy slurries. Since the medium being pumped only comes into contact with the tubing, the pump does not become contaminated. The tubing is easily changed so the pump is ideal for use on a variety of jobs requiring the quick and precise movement of diverse materials.



Fig. 46--(Right) Peristaltic pump used to fill the perfusion column.

Specifications

Priming: Self-priming and will hold vacuum when off.

Operation: Pump can run dry without damage

Capacity: From 3mL/min to 987mL/min (see model information chart)

Construction: High strength plastics and long-lasting alloy metals

Maximum Head Pressure: 57 feet of water

Maximum Suction Lift: 28 feet of water

Maximum System Pressure: 20psi continuous, 25psi intermittent.

Certification: All motors are UL listed

Rating: All gear motors are rated for 100% continuous duty

Installation Instructions

Power Requirements: Use power source (voltage & frequency) specified on the unit.

Wiring Connections: All wiring and electrical connections must comply with national electrical codes and local electrical codes.

Placement: Case enclosed model may be placed on any flat surface, assuring space is provided at the back of the case for air circulation through ventilation holes. The pump should be used in a dry location with an adequate supply of cool air. The ambient temperature should not exceed 25°C.



CAUTION: This unit should not be used outdoors or in hazardous locations.

SI-LANG2

Use the following color coded rollers with the appropriate tubing ID:

Roller Color	Tubing ID	mL per Revolution
Black	1/4"	3.5
Black	3/16"	2.1
Red	1/8"	0.84
Red	1/16"	0.21

Tubing Inspection

Inspect all tubing regularly and replace it if deterioration occurs. Use the following instructions to replace pump tubing.

Tubing Replacement

1. Disconnect the power to the pump.
2. Disconnect the suction and discharge tubes of the system from the tubing between the rollers of the pump.
3. Remove the four screws that hold the pump cover in place. Remove the cover.
4. Remove old pump tubing and discard.
5. Clean roller race, removing any particles that could damage tubing.
6. Position the roller bracket assembly as shown in Fig. 47-1.
7. Push the new tubing into the inlet port anchoring the tubing in grippers (Fig. 47-2) while rotating the roller bracket assembly.
8. Continue to rotate the roller bracket assembly, pushing the tubing into the roller race (Fig. 47-3).
9. Finally, insert the tubing into the outer port (Fig. 47-4), and replace the cover and screws.

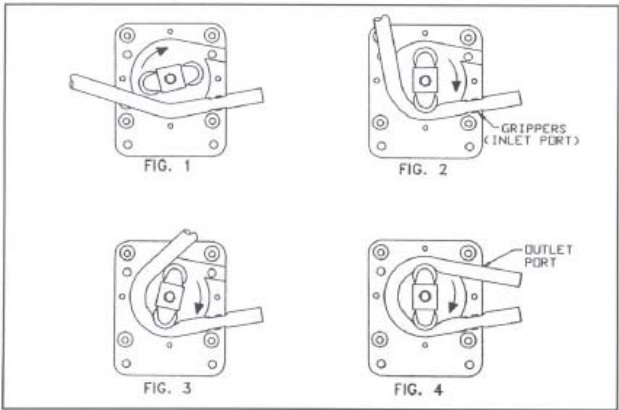


Fig. 47– (Right) Instructions for replacing the tubing.

**INDEX****A**

aorta 19, 27
aortic cannula 24

C

carbogen circuit 16
carboy 17, 18, 19, 20, 30
clean 30

E

ECG 24

H

heart chamber 10, 11, 21,
24
heart suspension unit 11
hypoxia 27

L

left ventricular pressure 21

LVP 21

M

MAP sensor 24

O

overflow tube 7

P

perfusion circuit 17
perfusion column 7
perfusion pressure 21
peristaltic pump 33
plumbing 12
pressure transducers 20

R

recirculating water bath
14, 30
reservoir 19

returns 3
ROCCAL 30

S

specifications 32

T

temperature regulation
circuit 14
temperature sensor 21
tubing 31

U

unpacking 3

W

water bath 19
water jackets 14

SI-LANG2

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

- Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.
- WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.
- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
- Goods returned for repair must be reasonably clean and free of hazardous materials.
- A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.
- Equipment which was built as a special order cannot be returned.
- Always refer to the RMA# when contacting WPI to obtain a status of your returned item.
- For any other issues regarding a claim or return, please contact the RMA department

Warning: This equipment is not designed or intended for use on humans.

* Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.

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