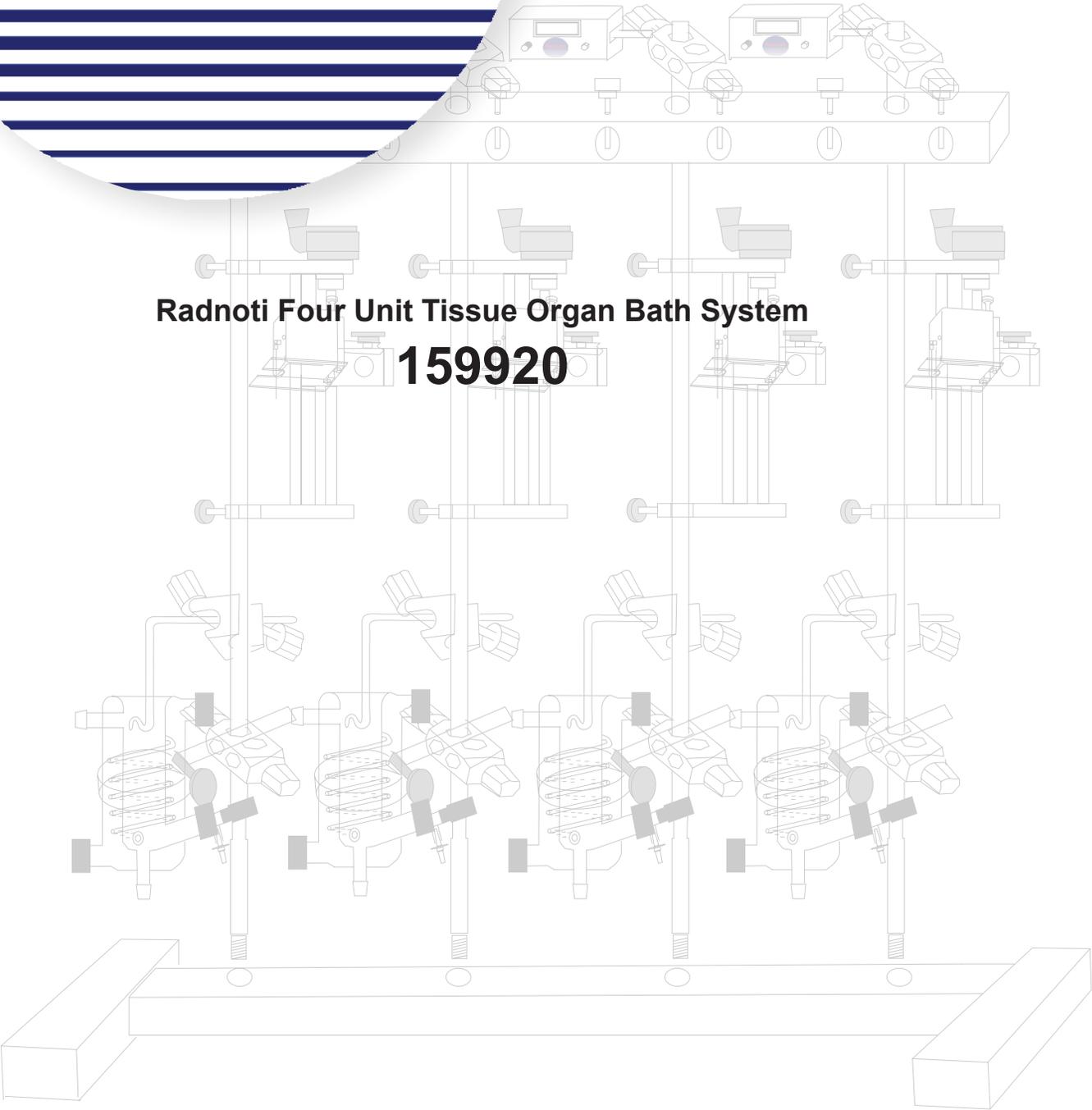




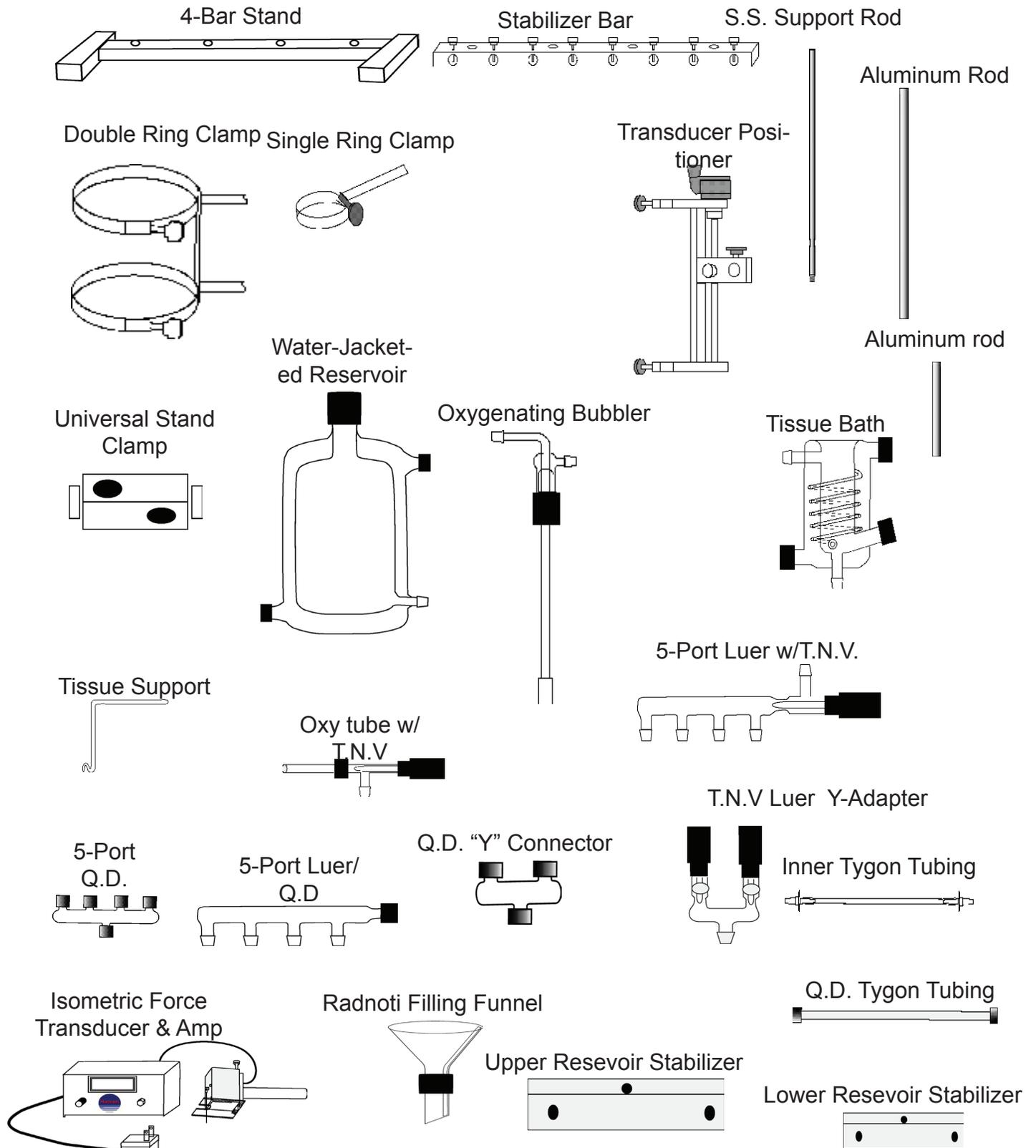
Radnoti



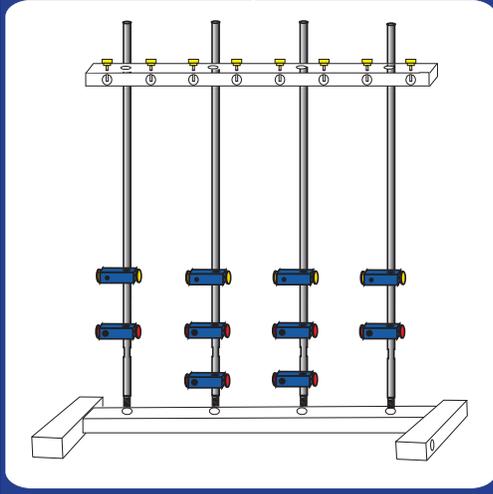
Radnoti Four Unit Tissue Organ Bath System

159920

Description	Qty	Part #
Base only, for 4-bar stand	1	159952-B4
Stabilizer for 4 bar stand	1	159950-C4
Rod 24" Long Stainless Steel	4	159950-24
Aluminum Rod 32"	1	159949-32
Aluminum Rod 10"	4	159949-10
Double Ring Clamp for Reservoir	1	120141-2
Single Ring Clamp (Tissue Baths)	4	159953-
Transducer Positioner	4	159906
Universal Stand Clamp	8	159952
Universal Stand Clamp (Glass)	8	159952-G
Water-Jacketed Reservoir 2 Liter	1	120142-2
Oxygenating Bubbler for 2 Liter Reservoir	1	120143-2
Tissue Organ Bath	4	1660-
Glass Hook	4	16017-
Oxygen Dispenser Tube with T.N.V.	4	16019-
Manifold 5 Port Luer with T.N.V.	1	120166
Manifold 5 Port Q.D.	3	120161
Manifold 4 Port Luer Q.D.	2	120164
Q.D. "Y" Adapter	1	120162
T.N.V. Luer "Y" Adapter	1	120168
Isometric Force Transducer & Amp	4	159901-A
Q.D. Tygon Tubing (Water-Jacketed)		
15"	6	120159-15
20"	6	120159-20
25"	1	120159-25
35"	2	120159-35
40"	2	120159-40
50"	2	120159-50
Inner Tygon Tubing (Perfusate, gas)		
15"	6	120157-15
20"	6	120157-20
35"	3	120157-35
Filling Funnel	1	120140-A
Upper Reservoir Stabilizer	1	159949-40
Lower Reservoir Stabilizer	1	159949-45

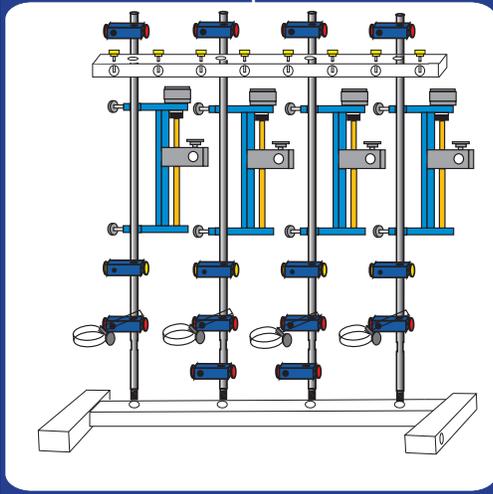


Step 1



Place Stand Base on a sturdy working surface. Thread the four 24" rods into the threaded holes on the base. Slide a Universal Stand Clamp on each of the two middle bars. Slide one Universal Stand Clamp on each 24" rod approximately 5" from the base. Slide one Universal Stand Clamp,(Glass) on each 24" rod approximately 8" from the base. Slide the stabilizer bar onto the four 24" rods approximately 20" from the base. Now assemble reservoir stand before proceeding to the next step.

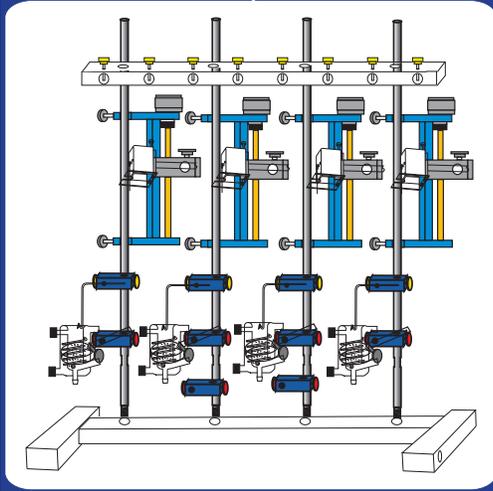
Step 2



Attach one transducer positioner on each 24" rod approximately 10" from the base. Slide one Universal Stand Clamp on each 24" rods above the crossbar to hold the transducer amplifier. Place a ring clamp for tissue bath in each Universal Stand Clamp located 5" above the base.

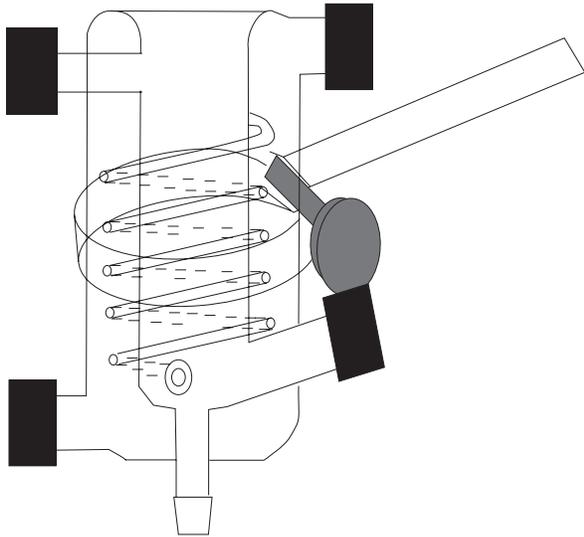
* When using Grass transducers and amplifiers, use the stand clamps with only one thumbscrew to hold the amp.

Step 3

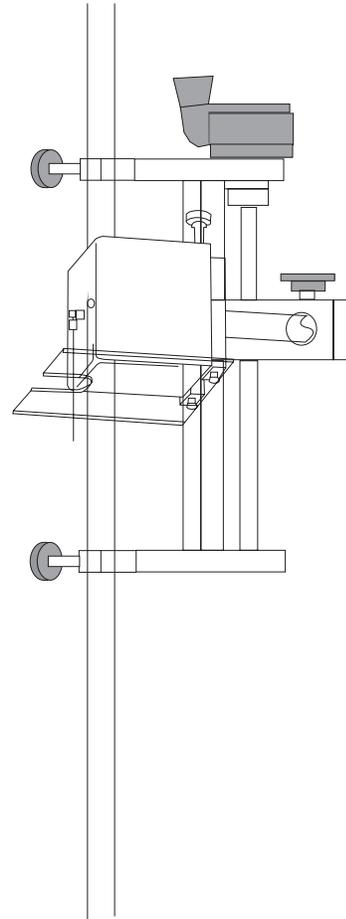


Place a tissue bath in each ring clamp. Slide tissue hook into the Universal Stand Clamp (Glass) located above tissue bath and align. Transducer amplifier is mounted using the Universal Stand Clamps, above stabilizer bar. Transducer head is mounted on positioner where indicated in (fig.1.2)

Open ring- clamp and insert tissue bath. Tighten clamp onto bath. (be careful not to over tighten)



Slide transducer head into positioner



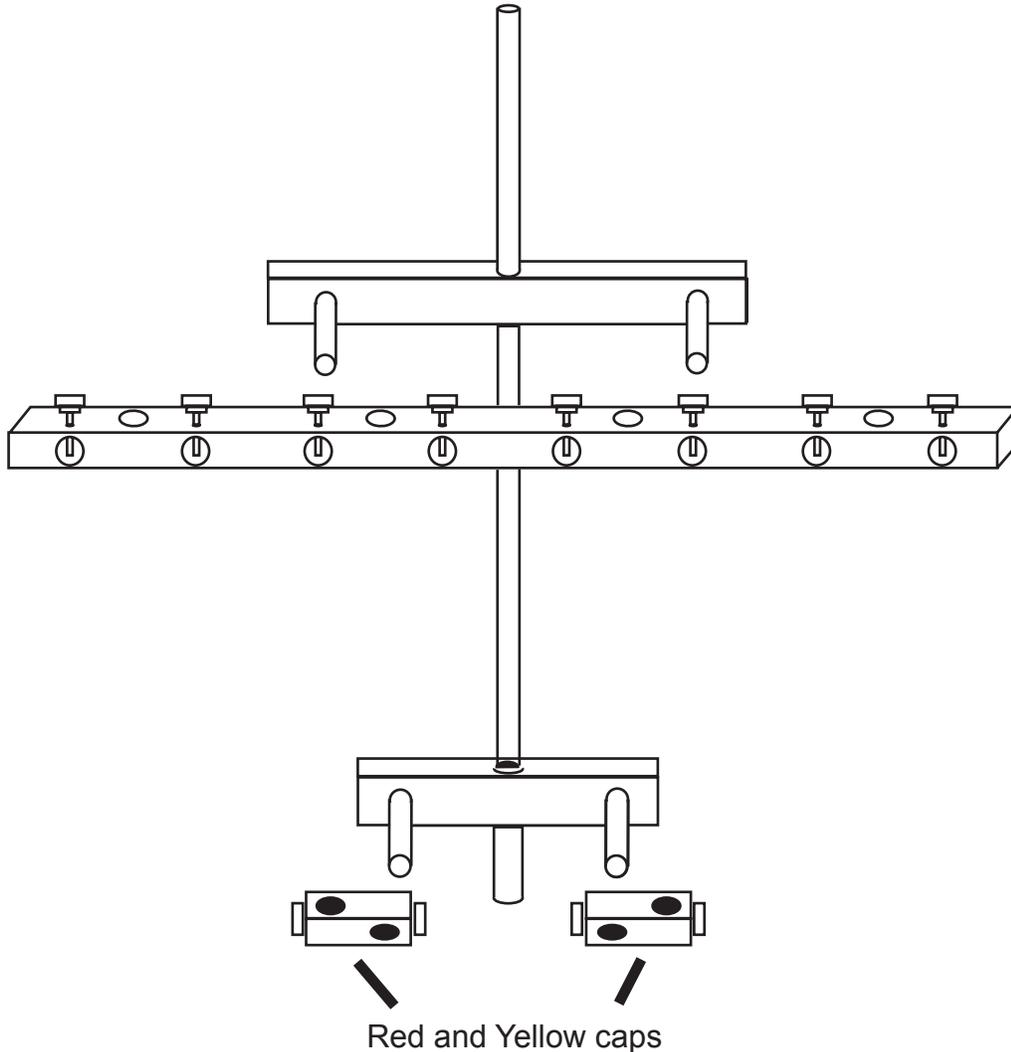
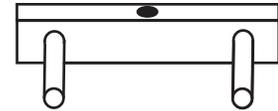
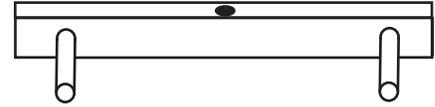
Reservoir Stand Assembly

Insert 10" aluminum rod in the side of both the Upper Reservoir Stabilizer and Lower Reservoir Stabilizer.

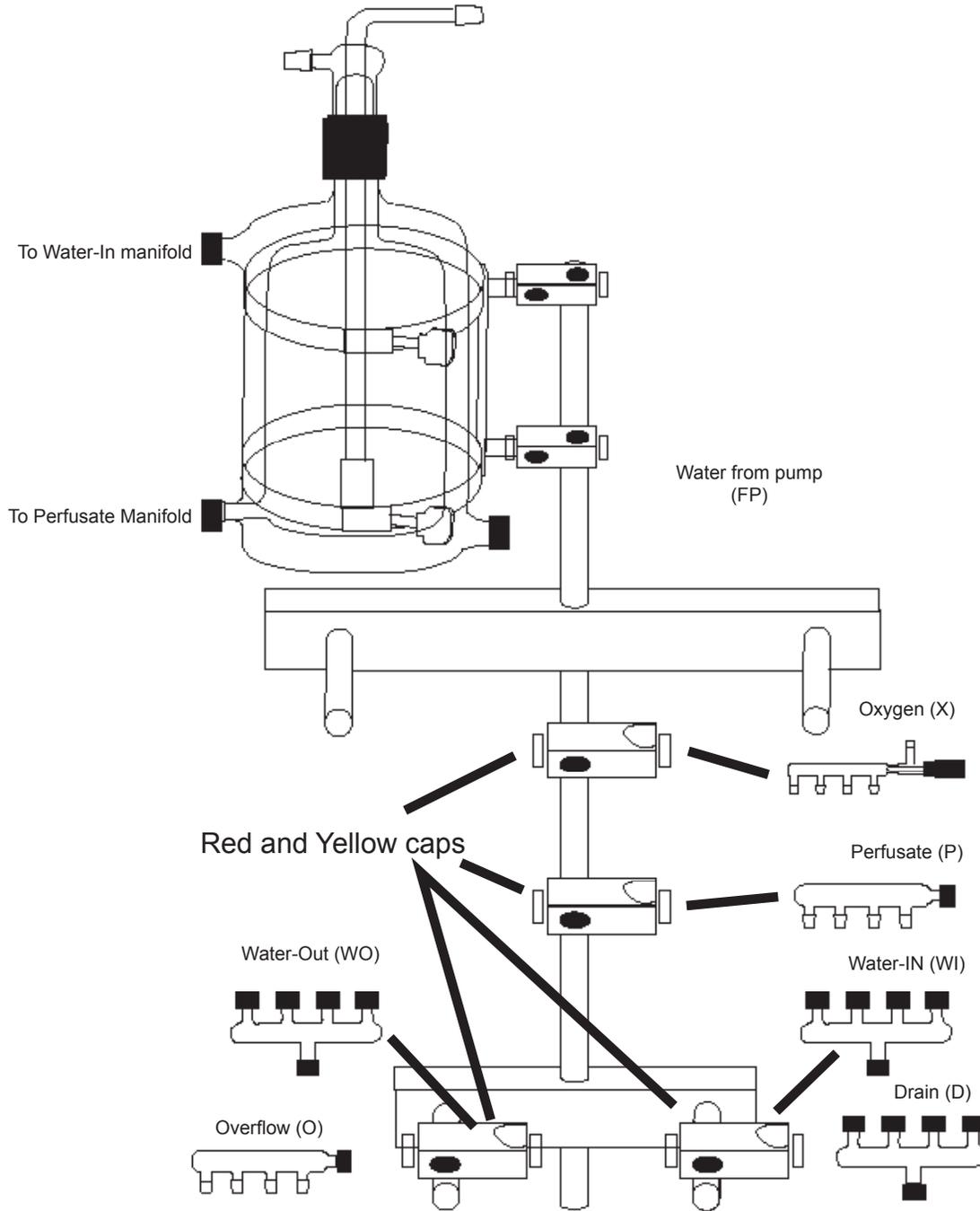
Slide the Lower Reservoir Stabilizer over the 32" rod. Next slide the Upper Reservoir Stabilizer over the 32" rod.

Line the Upper Reservoir Stabilizer with the Stabilizer Bar from the stand. Then line the Lower Reservoir Stabilizer with the two Universal Stand Clamps on the bottom of the base. Slide the assembly so it is in the middle of the base. The 10" rods should line up with the Stabilizer Bar and the bottom Universal Stand Clamp.

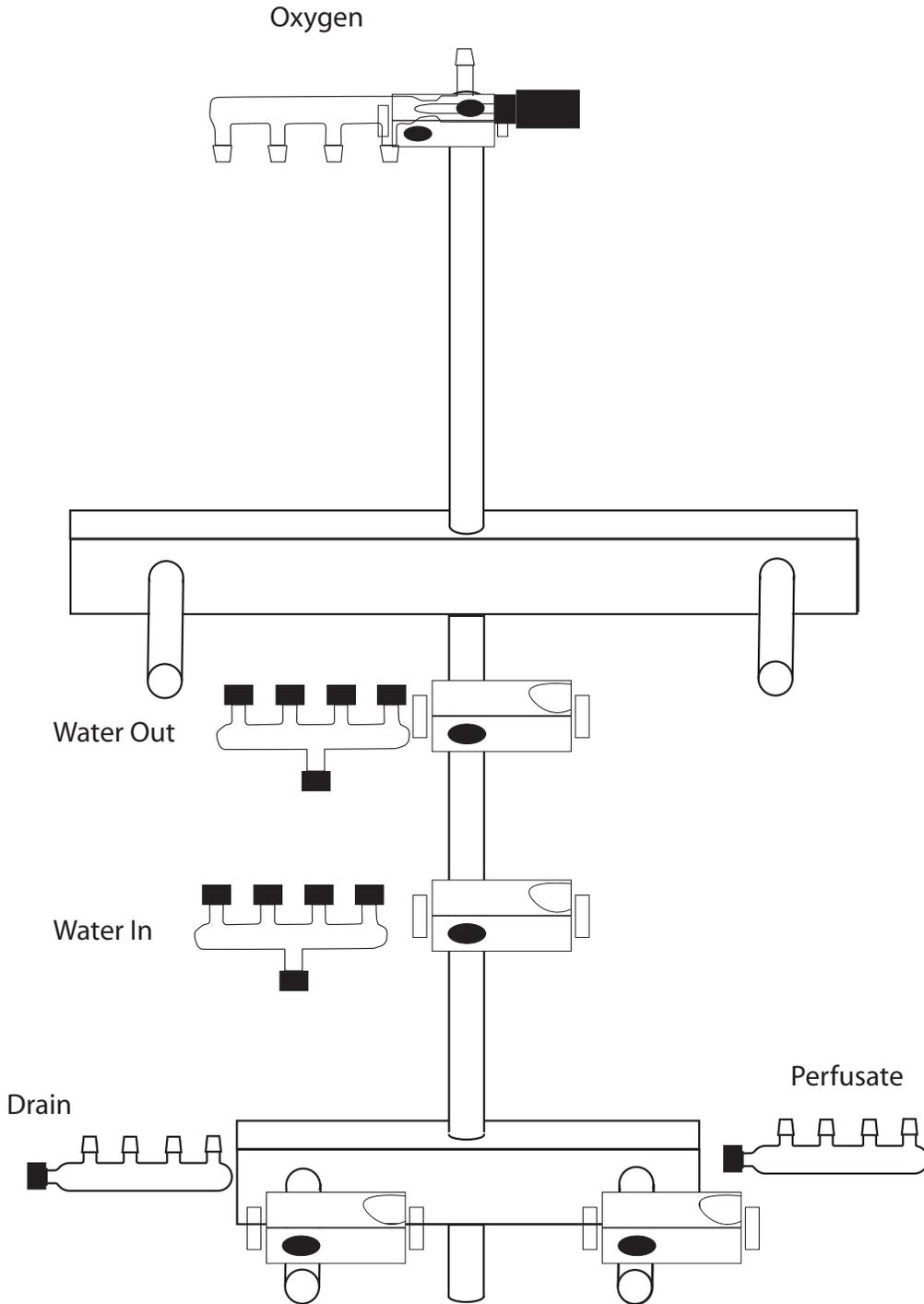
Place Universal Stand Clamps (Glass) on the 32" aluminum rod at 7" and 9" from the bottom. Place one Universal Stand Clamp on each of the 10" rods coming out of the Lower Reservoir Stabilizer. Slide two Universal Stand Clamps on the top of the 32" aluminum rod, spacing them to hold the reservoir ring clamp. Tighten securely



Reservoir Stand Assembly

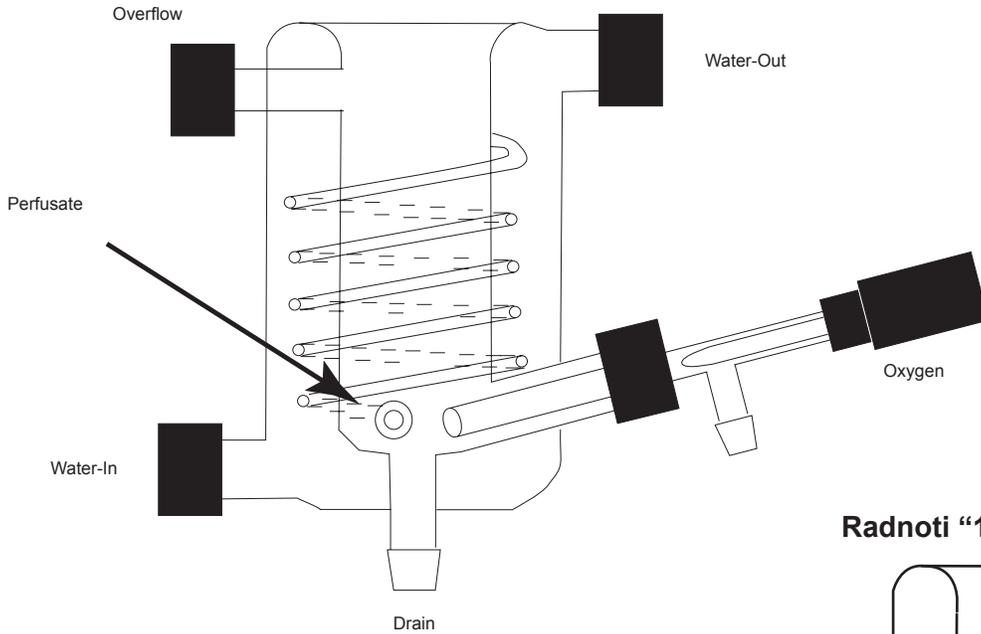


Reservoir Stand Assembly (Tissue Fluorometry)



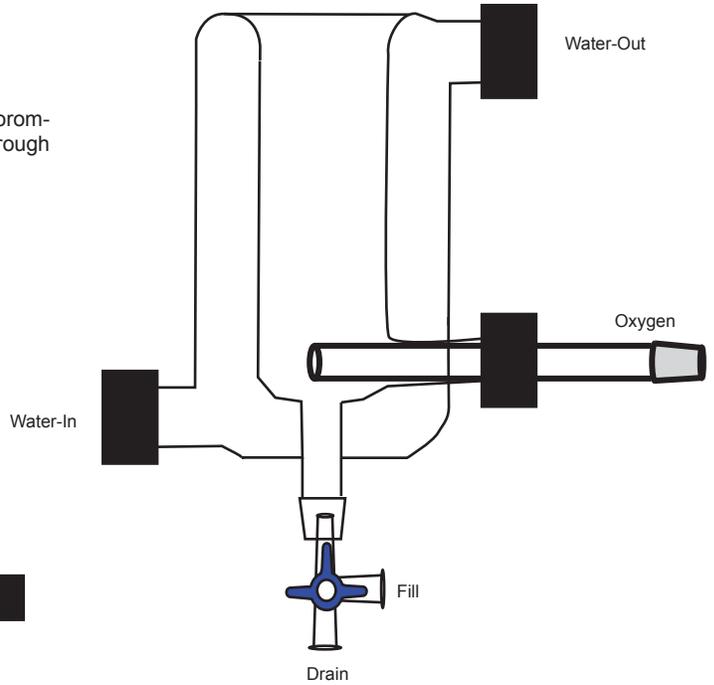
Plumbing for Baths

Radnoti 1660 "High Tech" Series Bath

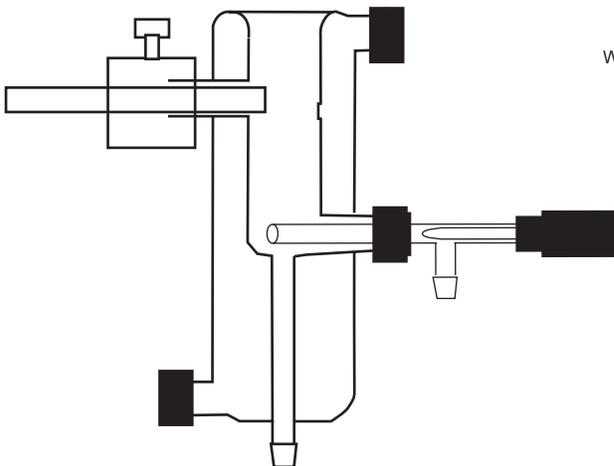


When using the 1583 series bath or Tissue Fluorometry there is no overflow and perfusate enters through a 3 way stopcock with the drain.

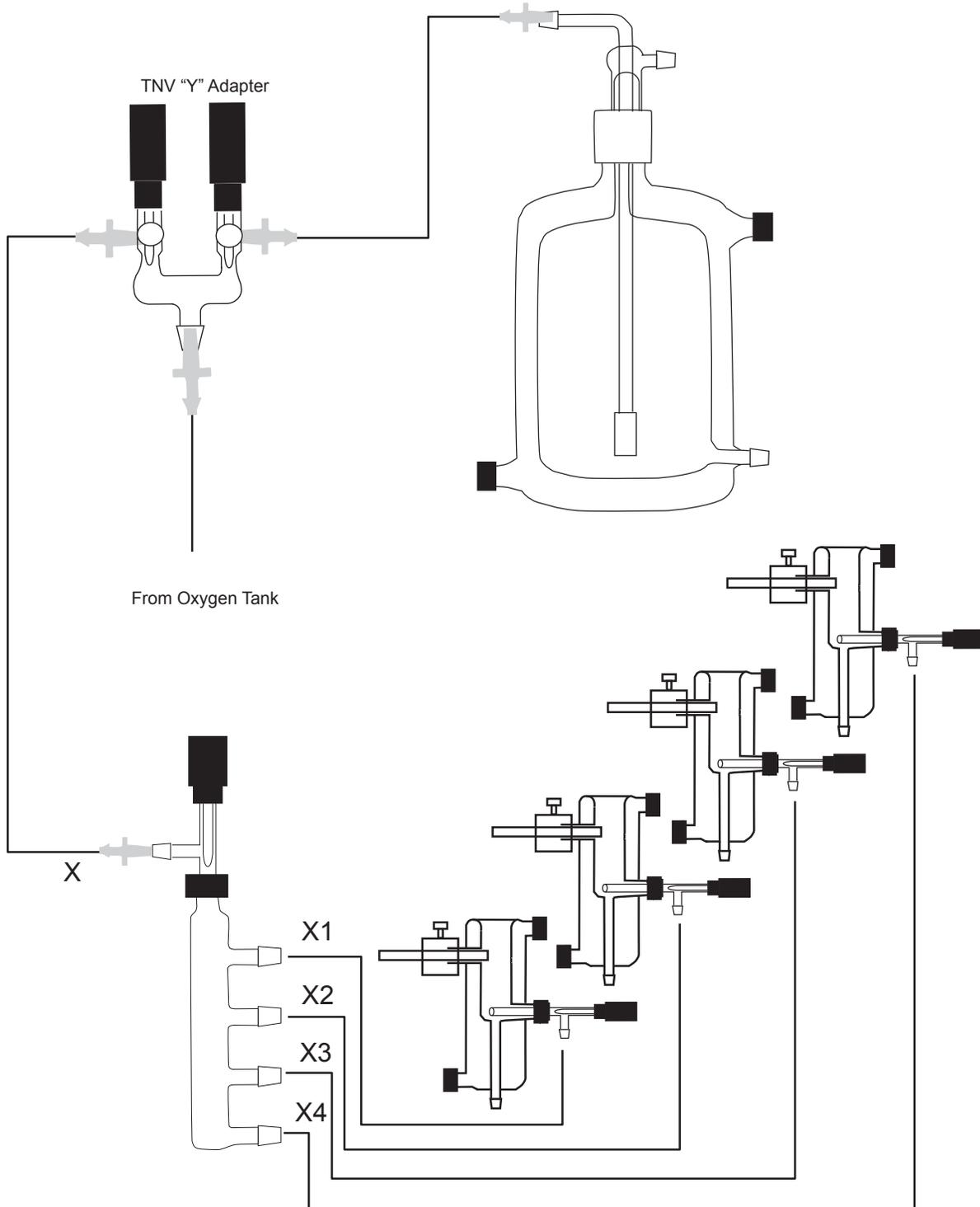
Radnoti "1583" Series Bath



Tissue Fluorometry Bath

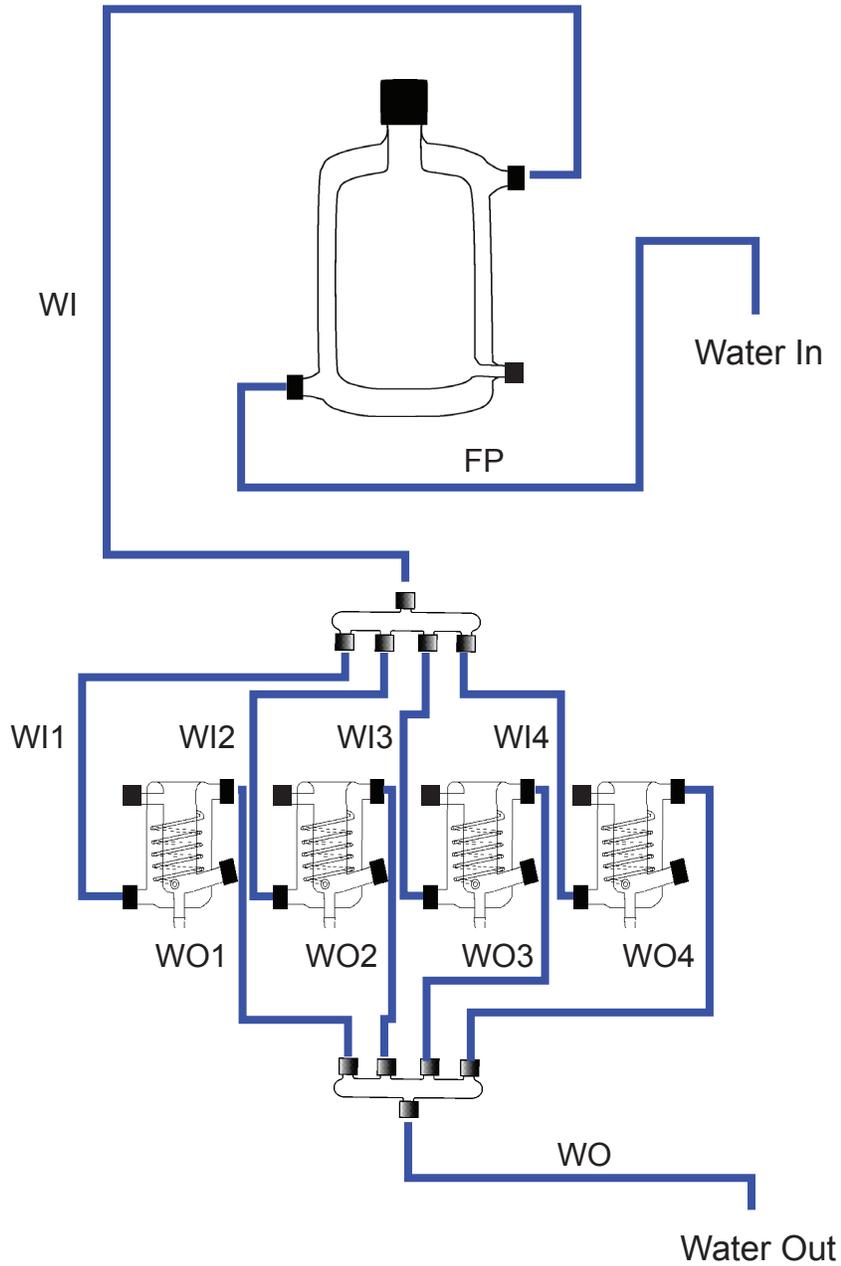


Oxygen Connection Chart

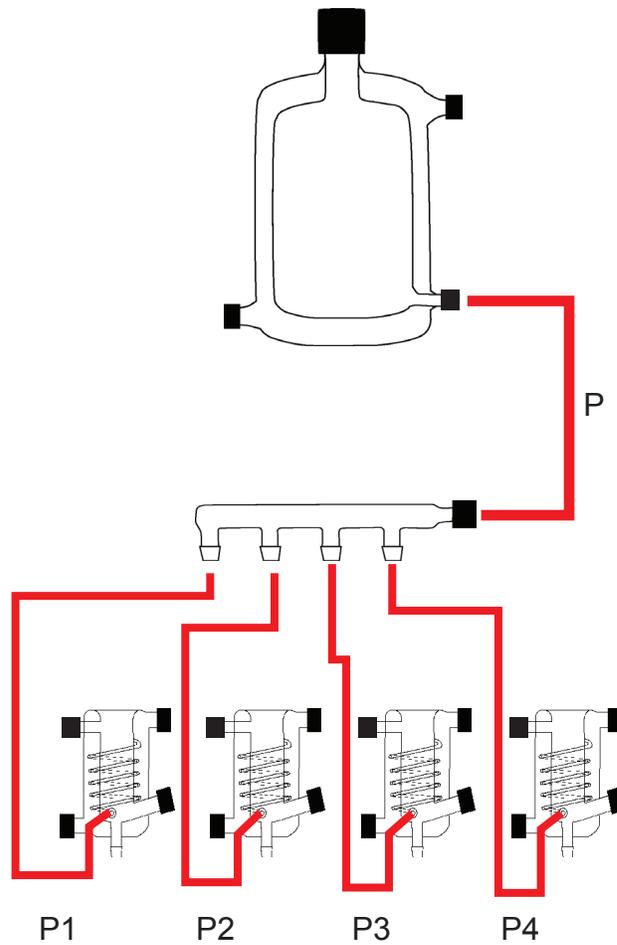


Note: The TNV "Y" adapter should be placed in the Stabilizer Bar (B)

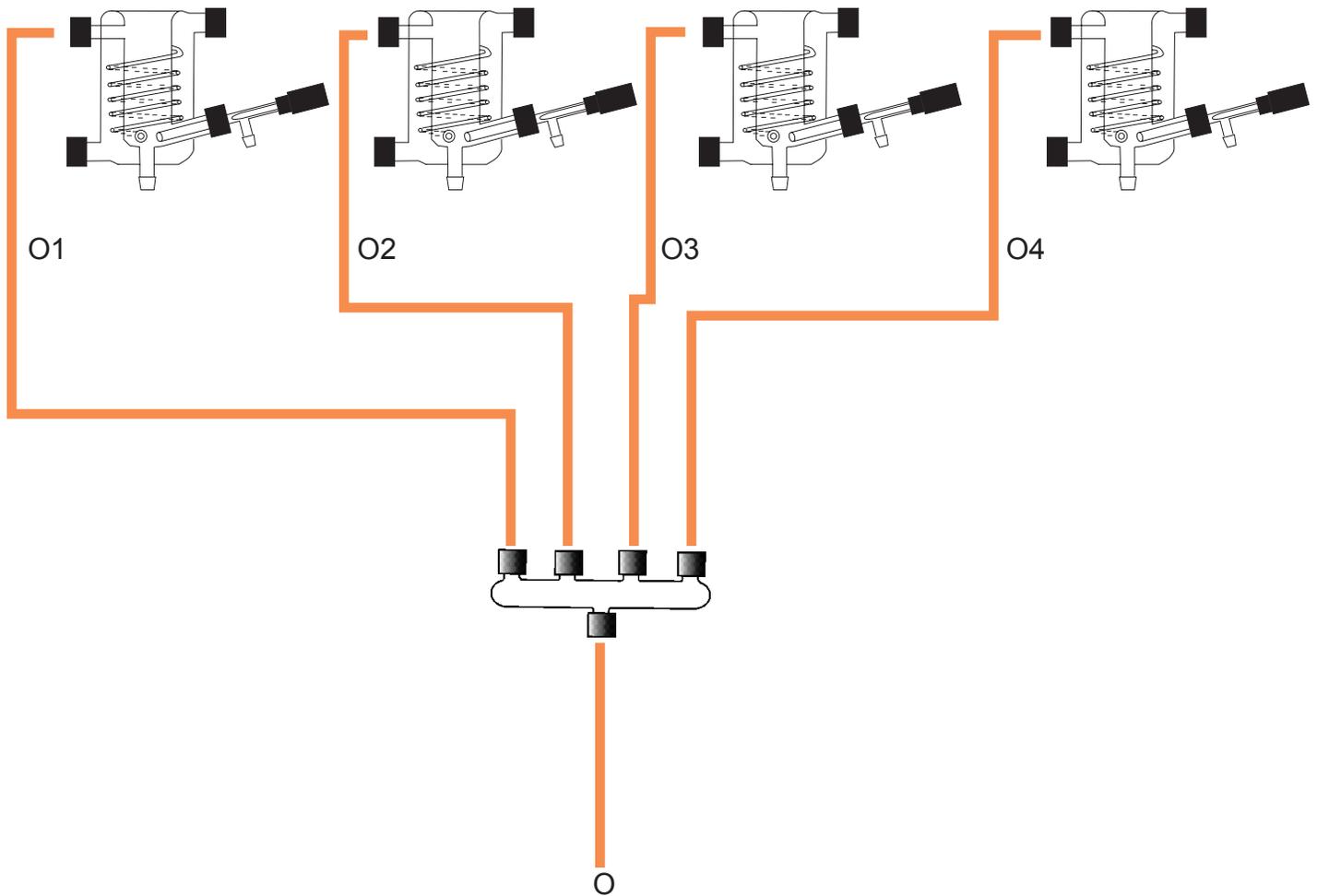
Water Jacket Chart



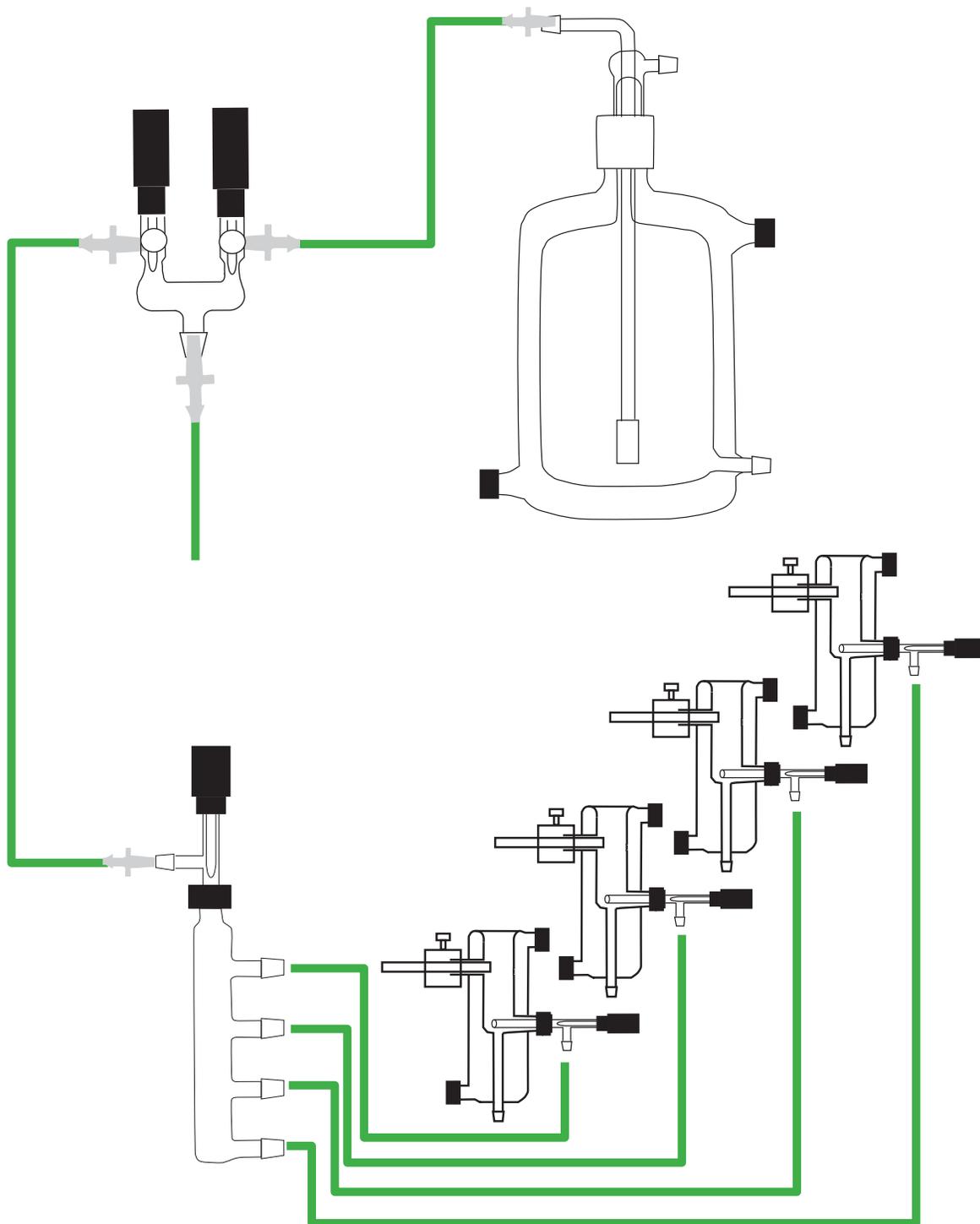
Perfusion Chart



Overflow (1660 Series Only)



Oxygen Connection Chart (Tissue Fluorometry)



Use of Radnoti 159901A Isometric Force Transducer

This transducer operates on the principle of converting picofarad capacitance changes into an amplified DC output voltage by means of a patented circuit (U.S. Patent Number 4142144). The transducer consists of a stiff beam suspended between two capacitor plates. This forms a differential capacitor. Using this principal, the beam can be exceptionally stiff, approaching the ideal of measuring force without motion. As an example, for a force of 2 grams, the beam deflection is a maximum of only 5 microns in either the 0.2 or 2.0 gram range mode. The linearity is within +1% with a high DC voltage output and freedom from drift.

Transducer Mounting

The transducer comes with an integral short, rigid mounting handle. To obtain maximum advantage of stiff beam characteristics of the transducer, it should be positioned and held in place with hardware that is as rigid as possible such that all the muscle forces are recorded (i.e. captured) and none are lost in extraneous deflections of the mounting hardware. The 159951 series of Radnoti Research Support Stands are the mounting hardware of choice. Their massive, durable construction will not deflect under load. The transducer itself should be positioned such that the output cable at the rear of the transducer ends up on the bottom (see Fig. 1 and 2).

Positioning the Muscle Sample

The transducer has a short tissue mounting rod with a groove that projects from the front (see figure1). One end of a non-stretching string or wire must be formed into a loop and fastened into the groove on the shaft. The other end of the string or wire is subsequently attached to the muscle preparation. The wire or string from the beam must be directed through the slot in the splash shield without the final setup has been completed.

Splash Shield

The transducer is equipped with a transparent, removable plastic shield which helps to prevent buildup of salts (i.e. corrosives) which may otherwise tend to accumulate on the transducer rod and migrate into the transducer circuit (see Fig. 1). In time, this salt buildup can deteriorate performance. The wire or string from the beam must be directed through the slot in the splash shield without touching the edges (see Fig. 1).

GENERAL INFORMATION

Amplifier / Power Supply

- a) The entire transducer is powered by a 12 volt AC wall transformer (see Fig. 2).
- b) There is no ON/OFF switch. The unit can remain powered, as shown on the digital meter, for the entire experiment.

Front Panel Controls

a) RANGE SELECTOR

The range selector switch has two positions: 2.0 gram and 20.0 gram (see Fig. 2).

- 1) In the 2.0 gram position, a 2.0 gram force produces a 5 VDC output.
- 2) in the 20.0 gram position, a 20.0 gram force produces a 5 VDC output.

b) OFFSET

The OFFSET control enables the user to set a zero output voltage for any pre-load value (see Fig. 2). It is essentially a "Tare Control" device since it balances out static loads such as the weight of the muscle preparation prior to contraction and the support wire or string.

Internal Controls and Adjustments

On the rear panel of the power supply are three small access holes to the trim ports (see Fig.3). The high and low gain trim ports are used to adjust the range. The right-hand trim port is factory set to deliver 5.0 VDC for 2.0 gram. The center trim port is factory set to deliver 5.0 VDC for 20.0 gram. Either can be adjusted to deliver more or less output as conditions dictate. The OFFSET adjustment trim port is located on the extreme left and has been factory adjusted to center the offset control. It also can be adjusted in situations where the front panel offset control does not have sufficient range. It can be used to correct for static loads equivalent to signals between +0.6v and -1.2v.

Electrical Output

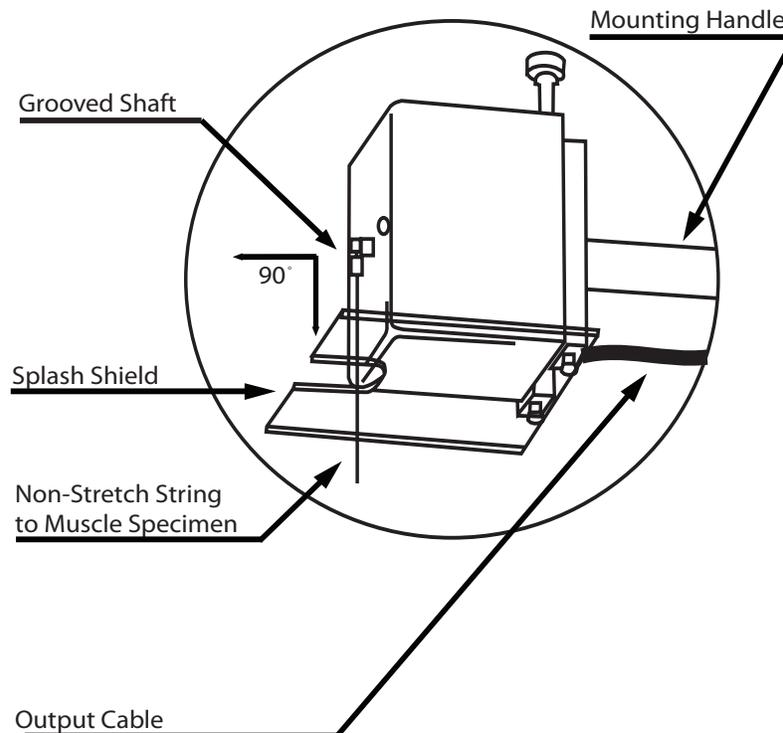
- a) A BNC jack on the rear of the amplifier is provided for output connections (see Fig. 3)
- b) Output voltage is 0 to 5.0 VDC depending upon the position of the range selector switch. *Output impedance is 2 kilo-ohms for direct connection to all recorders.
- c) The transducer is linear to 100% overload (4 or 40 grams, depending on setting).
- d) An internal frequency response jumper is factory set to 1000Hz
The jumper is located inside the chassis next to the trim pots. After unplugging the unit, the jumper can be moved to a second position forward towards the front of the amplifier. This will reduce the frequency response to 10 Hz for situations where signal dampening

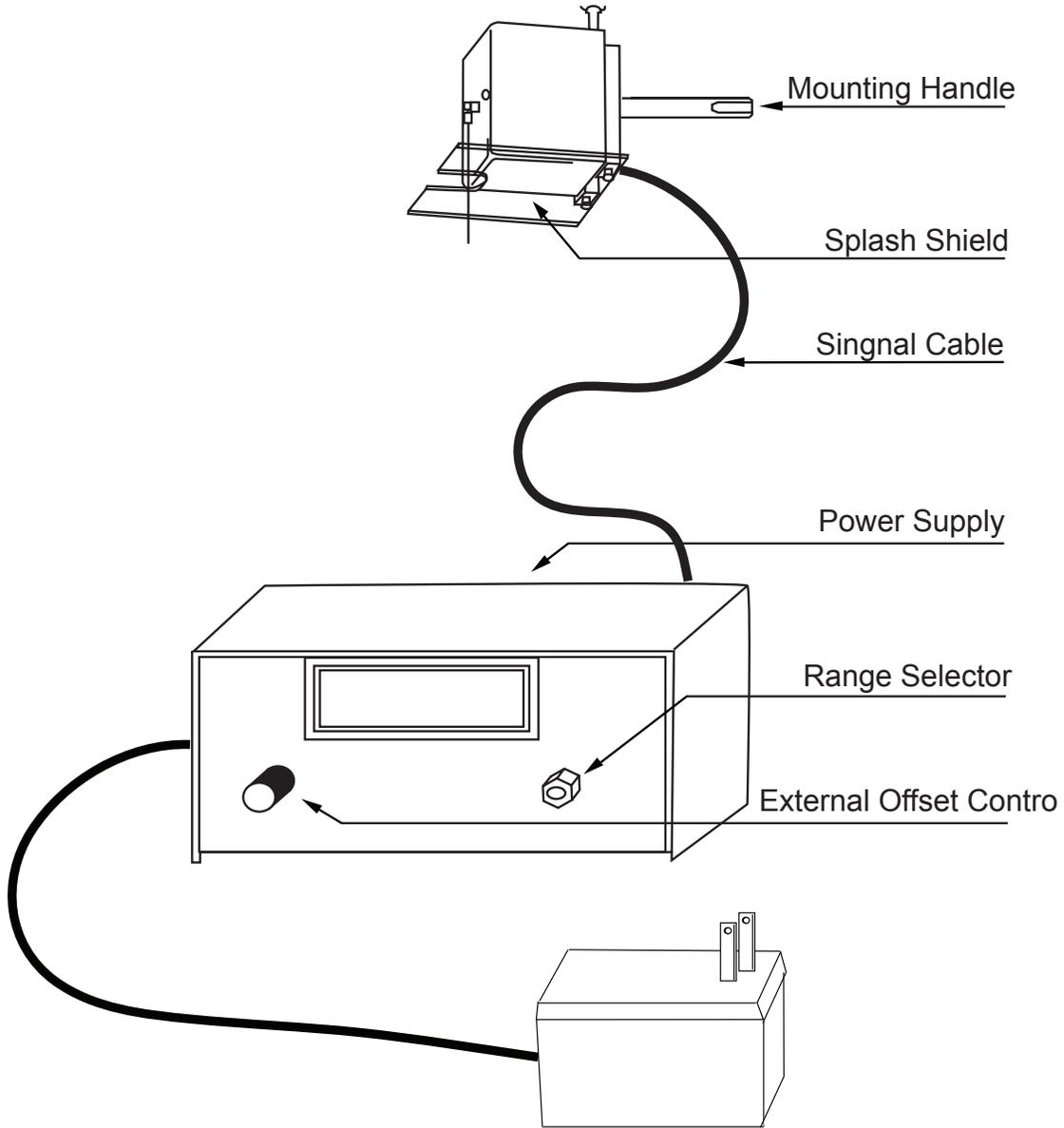
Maintenance and Storage

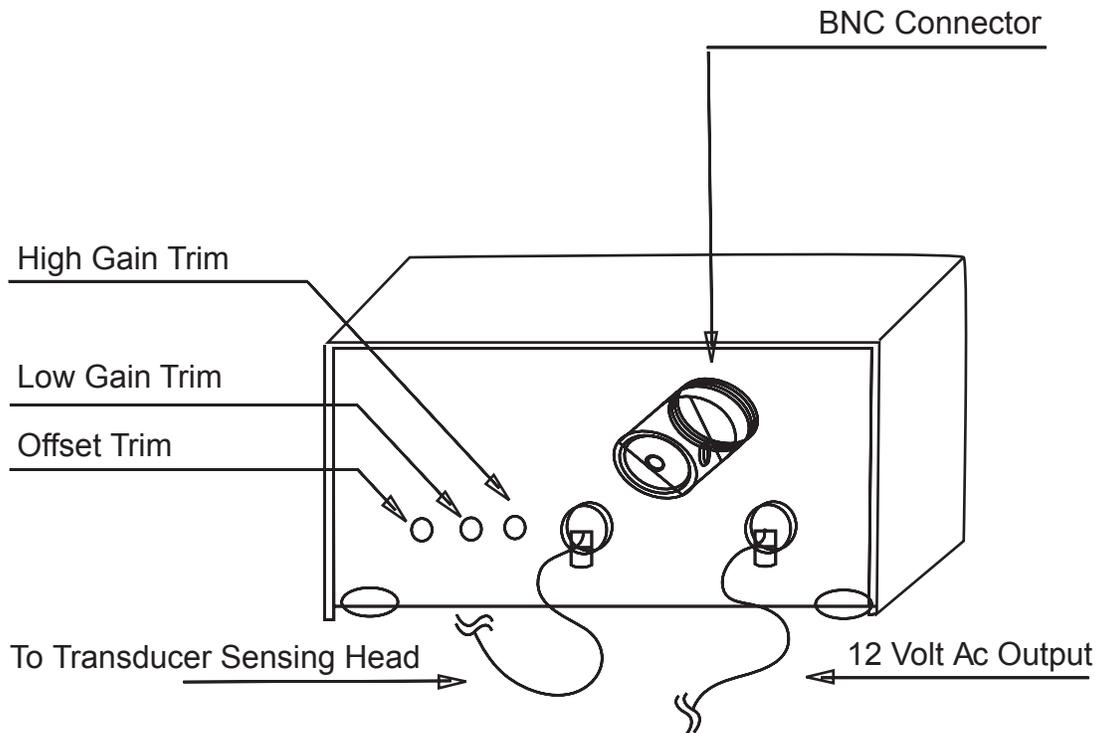
- a) No special precautions are required other than preventing corrosive solutions from entering the space where the output shaft exits the transducer.
- b) When not in use, store in a clean, dry place.

Serial Number

The serial number is located on the Power supply circuit board. Refer to this number in any correspondence.







RADNOTI 'QUICK DISCONNECT" INSTRUCTIONS

For Reference only

A	Slide Cap-With hole onto the water line. (Tygon tubing #120159)
B	Push the plastic sleeve insert into the end of the Tygon tubing with the flared end first. (Catalog #120160)
C	Be sure to use 5/16 x 3/16 inch Tygon tubing. (Catalog #120159)
D	<u>MOISTEN TIP OF TYGON TUBING</u> before insertion into the threaded glass.
	CAUTION: To avoid breakage, do not over tighten the screw cap.



(A) (B)

(D)

Plastic Insert

Replacement Part List:

Catalog No.	Description
120160	Plastic sleeve insert, 25/Pkg
120159	Tygon tubing 5/16 x 3/16 ID, 50 ft/min
160196	Drilled cap for W/J.Q.D.

RADNOTI TISSUE ORGAN BATH PRINCIPALS

Prior to any animal experimentation or use of animal or human tissue, researchers should discuss their protocols with their local animal care and use committee or institutional review board and biosafety (radiation, chemical, biohazard) committees to determine appropriate guidelines for their experiments. The material presented here is believed accurate but no liability is assumed for the use or misuse of said information.

Introduction

Isolated tissue and isolated organ preparations have been in use for over one hundred years, providing researchers with convenient biological models that exist without the systemic influences of the intact animal. Additionally, isolated tissue preparations can generally be run in groups of 2, 4, 8 or more, are more readily instrumented and can be more easily subjected to controlled changes in perfusate, oxygen drugs and other factors than intact animals. The recent development of transgenic animal models has extended the scope of isolated tissue and organ preparations by creating models that can express normal or pathological human or non-host animal genetic sequences (1). In turn, isolated preparations can permit molecular biologists to quantitate the physiological impact of the expression of these altered genetic sequences at the tissue and organ level.

Basic principles overview

To create a useful experimental isolated preparation one must construct a system that permits optimal performance of the tissue in a controlled environment that can be suitably instrumented to record changes in tissue function that are of interest to the experimenter. The researcher should therefore first determine (a) the basic hypotheses and specific aims of the research project (b) the species and the types of tissues that would be suitable research models (c) the kinds of experimental responses that might occur in these models (d) the methods, instruments, and statistics required to measure and analyze these responses. A careful review of the literature combined with a thoughtful analysis of the problem is paramount in designing a successful research project. There are numerous articles and reviews of isolated tissue and organ preparations available; a few overviews are listed.

The basic requirements for any isolated tissue preparation include provisions for temperature control and oxygen and substrate delivery. A water jacketed organ bath provides a stable and readily adjustable means of temperature control over the normal temperature range of most multicellular preparations. Substrates and other components required to maintain tissue function are usually provided in an aqueous solution, similar in chemical composition to that of the plasma of the donor animal. Oxygen delivery is usually supplied via saturation of the aqueous solution with a gas mixture, either through direct aeration of the solution or, when proteins, blood cells or other readily denatured membrane oxygenator or other non-turbulent delivery system. Additional tissue requirements may include electrical stimulation, changes in flow, pressure or distention, the presence of other cell types or tissues or other specialized features.

Instrumentation varies with the type of tissue and phenomena to be observed. For example, instruments are available to detect changes in force, flow or pressure, the release of chemical substances into the perfusate or alteration of perfusate characteristics, oxygen consumption or carbon dioxide production, changes in endogenous or exogenous optical sensors, electrophysiological alterations or literally any type of response the preparation may provide or the investigator may initiate. The responses can be recorded manually or on chart recorders, computers or other devices.

Selection of physiological buffer solution and manner of aeration

The buffer solution(s) chosen for the dissection and maintenance of the tissue will have a profound effect on the viability of the preparation. Whole blood from donors may be used, but since whole blood is generally in limited supply, it must be recycled, for example with an appropriate peristaltic pump through a membrane oxygenator, without denaturing blood proteins or causing erythrocyte damage. Whole blood may also contain biologically active materials, such as hormones and various cell types, that may affect the tissue preparation. Because of these limitations, most isolated tissue preparations use an artificial plasma-like buffer solution to maintain cell viability. Since almost all of these solutions are water based, water purity is a primary concern. The water purity should at least be the equivalent of double distilled water with organic contaminants removed. Achieving this standard used to be difficult, but with the advent of modern reverse osmosis systems or glass stills passing on water to charcoal and ion exchange resin filters, sources of purified water are now readily available with resistivity measurements in excess of 10MW. Some recipes for buffer solutions still add 0.1-5 mM EDTA (see Table 1) for chelation of heavy metals that may be added from salts or leached from metal tubing or other sources.

After storing the water in clean, non-leaching containers, the next decision to be made is which formulation of the buffer solution should be used. Reagents should be of high quality (the equivalent of USP or Analytical Reagent grade) and kept isolated from the rest of the chemicals in the laboratory to reduce the possibility of misuse or contamination. There are a number of widely used physiological salt solutions, such as Ringer's (5), Tyrode's (6) and Krebs-Hanseleit (7, modified as in 8, see Table 1) and their salt concentrations are often modified to resemble those in the donor's plasma (see Radnoti Isolated Perfused Heart Table 1). Since these solutions must be buffered, a choice of buffering agents must also be made. Bicarbonate buffers such as Tyrode's and Krebs-Hanseleit, which are based on those naturally occurring in mammalian blood, are effective when in equilibrium with relatively high levels of carbon dioxide, hence the use of 95% oxygen and 5% carbon dioxide gas mixtures to aerate them. The use of 95-100% oxygen rather than atmospheric levels of ~20% is to increase the oxygen content of the solution to compensate for the lack of hemoglobin or other oxygen carriers. In most of these solutions, phosphates (or sulfates) are also added. Besides adjusting the pH and increasing buffering capacity, the presence of carbonates, bicarbonates and phosphates aids in maintaining normal anion homeostasis. The restrictions imposed by bicarbonate buffers are the requirement for a mixed gas aeration and the adverse effects on solubility products created by

these anions. As an alternative, buffering capacity can be created through the use of synthetic buffers like HEPES or MES in place of bi-carbonates with 100% oxygen used for aeration. It is also possible to use these synthetic buffers in addition to bicarbonate, and then adjust the sodium balance appropriately to maintain osmolarity. The most common metabolic substrate is glucose, with pyruvate, lactate, and fatty acids also added. To aid in glucose metabolism, insulin may be added. Often salts other than calcium are kept as a concentrated stock in one container and calcium and glucose kept as a concentrated stock in another container and then diluted and combined just prior to use. This is done to reduce precipitation of calcium phosphate and retard bacterial growth by having hyperosmotic solutions. To maintain an appropriate oncotic pressure that will reduce edema, albumin, polyvinylpyrrolidone, dextran or other plasma expanders can be added. Note that the presence of albumin or other readily denatured or poorly soluble compounds will necessitate indirect aeration, such as that provided by a membrane oxygenator, to prevent foaming and precipitation. Always adjust the pH of the solution while it is at the bath temperature selected and when it is normally aerated.

Gas flow to the buffer is normally controlled with a two-stage regulator designed for the gas mixture utilized. Flow rates of 0.5-2 liters per minute and/or pressures of 1-2 psi are adequate for most preparations. The aeration stream can be controlled with a needle valve and should be a steady line of fine bubbles that do not cause a pronounced "boiling" effect in the bath. Higher pressures will only serve to increase evaporation, jostle the preparation and create noise in the force trace and may perforate glass frits used for aeration. For thin tissues with a large surface area and low metabolic activity (such as blood vessel strips or rings), aeration using room air may be sufficient; in these cases simple aquarium pumps are a cost effective replacement for compressed air tanks.

Selection of tissue donors

Sources of tissue may be from living or dead donors, depending upon the availability of the tissue type and the requirements of the experimenter. For tissues obtained from deceased donors, the type of tissue, the manner of death or euthanasia, the time elapsed between death and harvesting the tissue and the temperature that the donor's body is maintained at will affect the viability of the preparation. In the case of living donors, the selection and composition of anesthesia or euthanasia solutions should be made carefully to reduce the impact upon retrieved tissues. The presence of high concentrations of barbiturates, general anesthetics, ethanol, potassium or other substances used in or with the anesthesia or euthanasia may slow functional recovery, have unexpected long term effects or possibly cause irreversible damage. The species, variety, age and sex of the donor should be taken into account, both for the impact on physiological responses and for the fact that older animals have fatty deposits that make dissections more difficult.

Dissections in the field

For some preparations, such as those involving tissues from commercial processors, field dissections are generally a necessity. Therefore, the experimenter must be concerned with the duration of transport and its effect on tissue viability. A kit or container with the necessary supplies and instruments should be prepared before time and all appropriate paperwork be completed in advance. A trial dissection in the laboratory with a test or mock tissue should be used to evaluate the kit and procedures prior to actual dissections. Portable ice chests with locking lids and screw capped, large mouth containers are very useful to preserve tissues; they may have to be combined with air pumps or stimulators insure the viability of some preparations. The upside down ice chest lid can serve as a work table/ice tray. Eye loupes, magnifying eyeglasses or ring magnifiers can be serviceable replacements for dissecting microscopes. The on-site dissection may be simple or extensive, depending on the tissue isolated and the duration and mode of transport. The experimenter should protect against biological contamination of themselves and their research site caused by tissues, blood products or other materials that the experimenter may come into contact with during the isolation procedure. If national, state or local agencies are involved, consulting them ahead of time regarding legal forms or other formalities can save much time and insure that your presence at the procurement site will be well received. In a commercial slaughterhouse (OSHA) safety requirements may necessitate protective equipment, such as a helmet and protective eyewear and clothing.

Dissections in the Laboratory

If the dissection is performed or completed in the laboratory, a workstation should be set up that has a sink, running water and is located out of the general stream of traffic. A desk, bench or table with clearance for the experimenter's legs and a comfortable, adjustable chair with backrest are suitable; personal preferences will determine whether the chair is with or without wheels. Proper adjustments of chair height, lighting and magnifiers will significantly improve the quality of the dissection. The chair height and backrest should be adjusted to permit the easy use of a dissecting microscope or magnifier and allow the experimenter's forearms to be positioned on the table roughly at heart level. The level of magnification should be chosen to clearly distinguish areas of interest and the magnifying lens should have a good depth of field; a zoom lens is ideal. For greater ease of positioning, the dissecting microscope or magnifier can be mounted on a swing arm. The work area should be well lit, without glare, and a contrasting background may be used to aid in distinguishing the tissue. A small section of a plastic ruler can be placed in the dissecting dish (described below) to assist in measuring and cutting tissue samples. The use of fiber optic cables attached to lamps will reduce the amount of tissue sample heating.

The dissection bath is usually a low flat dish that is water-jacketed and aerated, permitting easy access to the tissue and visualization with a microscope or magnifier. The dissection is often performed at reduced temperature with the dissection bath kept at 4 0 C through the use of a refrigerated water bath or a container of ice. The reduced temperature is used to decrease tissue damage by slowing down metabolic processes occurring in the cell, thereby reducing the requirements for oxygen and nutrients, as well as retarding lytic processes. The dissection bath can be coated with a layer of silicone plastic (Sylgard[®]), to permit the tissue to be pinned to the bath's bottom. For dissection of small structures such as micro blood vessels, the dissection bath can be embedded in the table or positioned on a plastic block to allow convenient positioning of the fore

arms on the same level as the vessel floor, thereby steadying the arms more effectively. Tubular preparations such as blood vessels and hollow organs can be slid onto smooth glass, stainless steel or plastic rods which will serve as mounts to aid in dissection. A syringe attached to a plastic or metal cannula may be used to flush out the interior of blood vessels.

After appropriate anesthesia or euthanasia has been administered to the donor animal, the organs or tissues are generally removed. It is also possible to leave the organs inside the donor; a number of preparations, especially liver, kidney and intestine, can be set up with the major blood vessels cannulated and perfused with blood or physiological salt solutions. In either case, the host animal and the solution into which the sample is placed often are treated with anti-coagulants such as heparin. If blood clots are formed, they will clog the blood vessels in the organ and reduce the ability to perfuse the tissue, decreasing tissue viability. The presence of blood elements may also engender various inflammatory and necrotic responses in the tissue. Anticoagulants are not totally innocuous in that they have side effects that may affect cellular biochemistry and physiology. If the samples are to be removed from the donor, this ideally should be done quickly with minimal handling so that there is little damage to the sample. This ideal situation is modified by the anatomical location of the tissue in the donor, the anatomical attachments that must be removed, the degree of tissue, organ, neural, epithelial and vascular integrity required of the preparation and the final form of the preparation (whole organ, strips, rings, etc.). If tissue is removed from an anesthetized donor, the donor should be maintained in a physiologically normal state or in one of reduced metabolic activity (hypothermia), unless the experimenter is interested in creating a pathological state such as ischemia within the donor and/or experimental tissue.

During the surgery, the experimenter will find that the dissection is easier if the area around the tissue or organ is well exposed and kept as free as possible of blood or obscuring tissues through the use of saline rinses and suction in conjunction with retractors, gauze sponges or pins. Having sutures precut and instruments and sponges at hand greatly expedites dissections. The initial cut down and removal of tissue, whether in the field or laboratory, is often followed by a more rigorous dissection which can last for 30-60 minutes depending on the preparation. This is because it is often necessary to clean the tissue to remove unwanted cell types (epithelium) or debris (blood clots), to cannulate blood vessels or to cut the tissue into rings or strips that can be more easily attached to measuring devices.

The surgical equipment used for these dissections should be carefully selected and maintained separate from other equipment in the laboratory to avoid chemical or biological contamination, loss or misappropriation and subsequent loss or dulling of the instruments' points or tips. Having sutures precut and instruments and sponges at hand in a kit using a plastic box with dividers or a cutlery or desk organizer tray greatly expedites dissections. Many researchers use instruments made of high quality stainless steel for ease of maintenance, although carbon steel can be used when a sharper tip or edge is needed and instruments made of titanium and other metals, glasses, ceramics and plastics are available. Forceps and scissors should have tips sheathed with a soft plastic Tygon or silicone tubing to reduce damage. The instruments should be carefully and thoroughly cleaned by the researcher, preferably using low phosphate soaps, followed by thorough rinses in distilled water; if necessary, small brushes that are used only for instrument cleaning can be used on non-edged surfaces. Normally instrument sterilization is not required unless there is a problem of biological contamination/infection or a requirement for a sterile preparation. To sterilize instruments, wrap them and place the instruments in a covered tray in an autoclave for 30-80 minutes at 121½°C. Note that the direct heating of the fine tips or edges of instruments during flame sterilization may cause loss of tempering.

Once tissues are prepared, they may be stored for later use. Note that certain tissue samples, such as papillary muscles and especially smooth muscle strips and rings, can be stored in containers of chilled physiological salt solutions at 4 °C in a refrigerator for days and remain viable. Other preparations, such as the liver, can deteriorate in hours.

Selection and use of the tissue/organ bath

After the tissue or organ is prepared, it is placed in a bath, which is often customized for the particular type of sample used or experiment to be performed. Vertical baths require less space to set up than horizontal units; however, horizontal units permit the use of regular or inverted microscopes to visualize the preparation. The bath may be a simple water jacketed container with aeration tube and drain. The size of the bath should be selected to accommodate the tissue and whatever instrumentation must be inserted into the bath, with an attempt to reduce the bath volume to conserve drugs added to the bath without compromising the amount of solution required to sustain the tissue between washes. The bath should be securely mounted a rigid stand and should be easily adjusted up or down so that tissues can be inserted or removed. For a more precise control of fluid entry and exit, a warming coil can be built into the water jacket of the bath to adjust the temperature of the solution entering the bath and an overflow port can be placed on top of the bath. To reduce artifacts caused by aeration occurring during force measurements, the tissue can be positioned outside of the aeration stream; for very small tissue samples such as cardiac papillary muscle, a specialized bath with a remote aeration chamber can be used (9). The tissue may be mounted on supports (as described below) or tied onto cannulae made of metal or glass.

To simulate or replace neural stimulation or a pacemaker, tissues can be depolarized by the introduction of electrical current into the perfusate or into the tissue. If stimulating electrodes are required, field electrodes can be placed in the walls of small baths to conserve space, or field or contact electrodes may be attached to the tissue support. In certain cases, such as the isolated diaphragm, direct stimulation through the (phrenic) nerve can also be applied. For field stimulation, the tissue is placed between but not touching two electrodes, which may be formed into plates, rings, or wires. Successful field stimulation generally requires a field strength of 10-50 V/cm, depending upon electrode orientation (Chapter 16, 3). Field stimulation does not depend upon propagation through cells, while the use of contact electrodes does. As a comparison, contact electrodes normally require <5 V applied directly to the tissue (via surface or impalement electrodes). Therefore, the use of field stimulation will create a much larger demand on the stimulator output than direct stimulation. The electrical stimulation may be administered as discrete, repetitive single pulses (to replace or overdrive a cardiac tissue pacemaker) or given as a complex pattern called a train (to initiate smooth muscle

neurotransmitter release).

The pre-warmed tissue bath solution can be added from an external, jacketed reservoir or beaker, or passed through a pre-warming coil or water jacketed tubing prior to entering the tissue bath. If several solutions are used and the solutions are not expensive to formulate, manifolds with stopcocks or valves can be used to select different bathing solutions held in the remote reservoirs. The solution is removed from the bath through the use of a drain in the bottom of the bath. Drainage is provided either via gravity or suction, the latter created by a running-water aspirator or house or pump vacuum with a vacuum trap. If a solution must continuously flow through the bath, an overflow port connected to a vacuum or gravity drainage system can remove the solution. Drugs can be added directly to the bath, either via manual pipettes or through the use of syringe pumps or injectors.

A period of acclimatization is generally used to permit the tissue to adjust to its new surroundings. This period can vary from 10-15 minutes for an isolated heart to several hours for isolated smooth muscle, depending upon the tissue, storage or surgical procedures and experimental protocols. During this time, the tissue may be gradually stretched or undergo pre-treatment with selected agonists or antagonists (to limit or increase physiologic and pharmacological responses) or be exposed to probes or radio chemicals that are taken up into the tissues. This acclimatization period allows tissues to recover from the effects of cooling and/or surgical trauma and to wash out metabolites, anesthetics and other products used or produced during these periods. Tissue preparations normally have the bath washed out at timed intervals or before and after the addition of drugs.

Perfused Preparations

Preparations with one or more functional blood vessels present have the option of being perfused at either a constant pressure without recirculation, constant pressure with recirculation constant flow without or constant flow with recirculation. A variation of the non-recirculating, constant pressure system is when a cannulated blood vessel has one end secured, forming a blind sac (without flow through) that can be pressurized; this procedure is used for micro blood vessel studies. Perfused systems may be configured in a non-recirculating mode, where the solution only passes through the organ once, or in a recirculating mode, where the solution passes through the organ many times. In both tissues and organs, the non-recirculating mode is useful to remove (wash out) drugs added to the sample and also permits a fresh supply of solution to be available. Recirculating the solution is useful when the experimenter wishes to (a) measure the increase in a substance released at low levels by the organ, such as metabolites or neurotransmitters (b) add rare or expensive materials, such as radioisotopes, experimental drugs, etc., to the solution and allow the tissue to equilibrate with them (c) limit the amount of solution required when the volume of solution necessary to maintain the organ in a single pass system would be excessive, as in a liver preparation.

There are drawbacks and benefits to either constant flow or constant pressure systems. Constant flow preparations are easier to monitor for changes in the resistance of blood vessels because with a constant flow rate, any constriction or dilation of the coronary vessels is revealed as a change in pressure, which can be measured with a pressure transducer. The production and release or efflux or uptake of substances is easier to calculate with a constant flow system. Constant flow systems also permit the addition and titration by syringe pump of small amounts of concentrated drug solutions that are then diluted. When added to the perfusate stream, thereby eliminating the necessity for large remote reservoirs of expensive drugs at preset concentrations. Limitations of this technique are requirements of high drug solubility, good mixing and solvents that are innocuous over the concentration range utilized. A drawback of the constant flow system is that if the internal requirements for nutrients in the tissue are not met at a set flow rate, the tissue or organ cannot compensate for this shortfall through vasodilatation.

Measurements of changes in vessel diameter in constant pressure systems require flow measurements, generally taken manually via a graduated cylinder, or with drop counters, fraction collectors, flow indicators or image analysis, magnetic or ultrasonic flow probes, the latter three types of instruments more expensive than pressure transducers used for constant flow systems. Drug titrations in constant pressure systems require the use of pre-mixed solutions in reservoirs. Constant pressure systems can require the repositioning of the elevated reservoir to maintain a constant pressure head, or the use of a refilling pump or pressurized refilling system. In a constant pressure system, the vessels can dilate and increase the total amount of perfusate flowing through the tissue or organ, thus satisfying changes in tissue oxygen and substrate demands. A variation of the non-recirculating, constant pressure system is when a blood vessels may have one end secured, forming a blind sac without flow through that can be pressurized; this procedure is used for micro blood vessel studies.

Measurements of tissue and organ activity

When the experimenter is first developing or establishing a preparation, it is always wise to have a series of positive and negative controls to determine how well the preparation is functioning, in part or in whole. These controls may be mechanical, pharmacological or physiological in nature. For example, acetylcholine has been used to vasodilate isolated vessel preparations (that have tone) to determine the presence of functional endothelium that can generate nitric oxide. The stability of the baseline activity, the response to agonists in the presence and absence of antagonists, the effective dose response range, onset of the response, the effect of stretching, oxygen consumption and carbon dioxide production can be used to determine how well a preparation is functioning and how it compares to another preparation. These controls may be repeated at the beginning, middle and end of the experimental series to monitor changes in tissue or organ performance. The use of standard controls also permits quality control and trouble shooting of the preparation once the protocols have been selected. Included in these tests should be the administration of solvents such as ethanol or DMSO over the concentration range used.

To measure muscular activity, the organ, muscle strip or ring is attached by wire or silk suture to a force transducer which converts the force generated by the muscle into an electrical signal that can then be detected on a chart recorder or a computer based data acquisition system. The

muscle is usually pre-loaded with a weight or pre-stretched using a tissue tensioner or micrometer. The wire or suture attaching the muscle to the transducer should not touch the bath walls and should be in line with the transducer. The force transducer may make an isometric or isotonic measurement. In an isometric measurement, the muscle length remains constant as force changes while in an isotonic system the muscle shortens against a constant force. Isometric measurements, which are measured as grams or millineutons are more commonly used in pharmacological experiments; isotonic measurements made at high speed can be used to measure shortening velocities (10). Force measurements can be converted to stress measurements, permitting the experimenter to control for the differences caused by the size of the preparation and its effect on force output (11). The output range of the transducer should be selected to optimize the size of the signal without plateauing (cropping) the maximum response. The frequency response of the transducer can be adjusted to dampen external vibrations and motion artifacts (noise), but this adjustment may reduce the ability to detect rapid force changes.

There are other measurements of muscular activity. A blood vessel or intestinal smooth muscle can be cannulated and when solution is delivered under

constant pressure or flow, a constriction of the vessel can be measured as a change in flow, pressure or visually as a change in diameter, the latter which can be recorded on videotape from a camera or via image analysis equipment. Electrical activity can be measured in muscular (and also neural) preparations through the use of glass microelectrodes inserted into the preparation, impalement or surface electrodes on the tissue or inserted into the bath media.

By equilibrating radioisotopes with the sample, the experimenter can follow drug metabolism, the formation of carbon dioxide, the formation and degradation of second messengers such as inositol phosphates and ion fluxes (^{45}Ca , ^{86}Rb , etc.) into and out of the tissue. These studies may be end point assays, in which the sample is washed out and/or extracted at a given time, or performed on a continuous basis, using fraction collectors or in-line detectors. The tissue may also release chemicals, produced by or stored in its cells, into the bath solution. The presence and identity of these chemicals can be measured by assaying the solution, either continuously in situ or as aliquots removed from the bath. Ion selective electrodes that detect changes in pH, anions or cations, nitric oxide, carbon dioxide or oxygen can be inserted either directly into the bath or be placed in flow-through adapters that are inserted into the perfusion tubing that is used to add or remove solution from the bath or sample. In a similar fashion, potentiometric electrodes can be used to determine the release of catecholamines and their chemical identity. A number of biochemical and molecular biological techniques are applicable to isolated tissue systems, such as 1 and 2 dimensional gel electrophoresis, Western blots, RT-PCR, etc. Optical methods for determining intracellular ion activities with fluorescent indicators have become quite popular, and the production of free radicals can be determined through bioluminescent assays that introduce luminol or other agents into tissues. Both vertical and horizontal baths can have optic ports to be used with microscopes or with fiber optic cables placed in line with the tissue samples to permit these measurements to be made.

A classical way to detect the release of chemicals from tissues is the bioassay. A bioassay for released substances can be performed by exposing another tissue to the released chemicals, causing that second tissue to respond by contracting or causing another type of easily measurable response. The second tissue may be in a standard or specialized bath and can be pre-treated with agonists or antagonist to modify its response characteristics.

Automation of isolated organ and tissue baths

From the advent of isolated tissue preparation, it was feasible to run several preparations simultaneously. The availability of computer A/D systems and simple robotics allows these isolated preparations to be partially or completely automated. With these systems experimenters can automatically drain and fill baths, adjust tension, add drugs and record measurements. Data can then be processed using generic or specialized analysis, graphic and statistical software. This significantly reduces the amount of time required to perform experiments and process data.

Limitations of isolated tissue preparations

Due to the damage occurring during removal of sample and the difficulty in exactly reproducing the normal environment of the sample when it's inside the host, tissue and organ preparations will not last indefinitely and their response may not be identical to in situ or in vivo preparations, To prolong the useful life of tissues, experimenters may use blood and blood proteins in their solutions, together with other chemicals. However, this has drawbacks in terms of expense, release of materials from damaged erythrocytes and other factors.

If a tissue is not perfused through its blood vessels or is not inherently porous, the delivery of oxygen and nutrients is restricted by diffusion. It has long been recognized that in tissues with high oxygen and nutrient consumption, such as cardiac muscle, a hypoxic core may exist if the tissue is $>0.5\text{mm}$ thick. If the tissue is not thin, the requirements for nutrients and oxygen must be low, as in the case of smooth muscle. It should be noted that the delivery of oxygen can be limiting in highly active tissues like heart and liver, even if these organs are perfused through their vascular bed. In perfused organs or tissues, oxygen consumption can be determined through the use of oxygen electrodes placed in the perfusate stream before and after the tissue. Other indicators for oxidative state are the mitochondrial redox state, as determined by NAD(P)H fluorescence (Chance, 1960). Other problems may arise due to imbalances in oncotic pressure or trace elements caused by the use of synthetic media, overstretching or mechanical damage to cell layers due to mechanical attachments, photo bleaching or metal chelation that occurs when indicators are used and numerous other confounding problems. When used with these limitations in mind, isolated organ and tissue preparations will continue to be very useful screening and research tools.

Glassware Maintenance & Post Experimental Cleanup

Post Experimental Cleanup

After the experiment has been completed, the experimenter should take care to scrupulously clean the equipment. It is important to remember that the solutions that can sustain the heart and muscle will also provide excellent media for bacteria. The cleaning procedures will be dependent upon the types of chemicals and biological materials that are being used, the types of measurements that are being made and what substances can interfere with those measurements and the frequency of the use of the equipment and number of operators involved. Non-phosphate soaps are preferred, since insoluble phosphates can form from calcium and magnesium in physiological salt solutions. Note that bactericidal soaps may contain iodine or other materials which can affect isolated tissues and cells. Cleaning supplies and equipment, such as brushes, should be used only for cleaning this glassware and not used for other lab cleaning procedures. Questions and procedures noted here should be adjusted in accordance with your licensed procedures and the recommendations of your safety personnel.

Shared equipment is the most difficult to maintain properly. In order to maintain equipment properly, it is generally best (1) to assign the maintenance or the oversight of the equipment to one individual, who will monitor equipment and maintain cleaning supplies (2) to have written protocols posted with the equipment (3) to have a logbook where cleaning dates, as well as notification of problems, suggestions, etc., can be recorded.

Often overlooked as a source of contamination is the water circulator supply. This should be kept clean and the bath rinsed and solution changed to reduce precipitate build up. Covering equipment to reduce air borne contamination from microbes and spores is useful. Note that when baths are used intermittently, the lack of frequent cleaning and the lack of solutions rinsing out bacteria that are deposited in the tubing may result in a contamination problem when the system is finally used. A convenient rule of thumb for testing for contamination in preparations that you have found reliable is that two consecutive experimental failures that are not explained by an obviously damaged sample, poor surgical or dissection techniques or solution problems may be caused by bath contamination.

Glassware

Much of the Radnoti apparatus is borosilicate glass, which can be cleaned with a wide range of soaps, ethyl alcohol, dilute HCl or HNO₃ (0.1 M) or other solvents. Extensive flushing with distilled, deionized water to remove all traces of the cleaning agents and salts is recommended. Large glassware, such as reservoirs or assemblies can be flushed in place, but care must be taken to thoroughly clean aerators, stopcocks and associated parts. Aerators should be blown dry using gas or air at the final water rinse. If acid is used, the runoff water should not be more acidic than the normal water pH. As with the use of any chemicals, proper protective gear and training are essential to reduce personnel hazards and experimental and environmental contamination. Heated acid or chromic acid is generally not recommended due to personnel hazards and possible heavy metal contamination of the system.

If very lipophilic substances (prostaglandins, ionophores, certain dyes, etc.) are used, rinses with ethyl alcohol or the most appropriate organic solvent can be used first, but this will necessitate thorough cleaning afterward to remove any traces of the organic solvent.

Use of toxins, biohazard materials, and radiochemicals can present considerable complications to a generalized cleaning procedure. Having an apparatus and a contained area dedicated to these procedures reduces problems. Diluted bleach can be used on glassware, but must be rinsed extensively. The use of disposable tubing and stopcocks will assist in cleanup, as will scheduling a run of these procedures, rather than intermittent experiments, if non-dedicated equipment must be used. Glassware can be sterilized but all fixtures, such as aerators, stopcocks caps, etc., should be removed prior to sterilization.

The glass aerators can be cleaned with water, or dilute acid if clogged. The use of water or gas under high pressure can result in damage to the glassware and personnel and therefore is not recommended. After a general soap and water rinse to remove soluble materials, cleaning with 0.1M HCl or 0.1 M HNO₃ for several hours or overnight, followed by an extensive water rinse, will usually remove most contaminants. If this does not work, 1 M acid can be tried. Because the glass frit filaments are thin, high concentrations of acids, or especially alkalis, can destroy them and are not recommended.

Non-glass items

Initial cleaning of non-glass items should be with aqueous soap solutions. Depending upon the chemical resistance of the materials, the use of other solvents, cleaning procedures or sterilization may be possible. Areas and items to be especially well cleaned are the aerator, tubing, syringe ports, cannulae, pressure transducer fittings, septa, balloon, along with other catheters, and electrodes (oxygen, pacing, ion selective, etc.). Tubing should be inspected at the pump head for wear. Note that the interior of tubing can gradually be roughened during use and the abraded areas will form sites for bacterial growth. Tubing should be a high grade with low plasticizer leaching. Note that silicone tubing is very permeant to gases, so it should not be generally used to transport gassed solutions.

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