INSTRUCTIONS FOR VESTAVIA SCIENTIFIC MICROPERFUSION SYSTEM

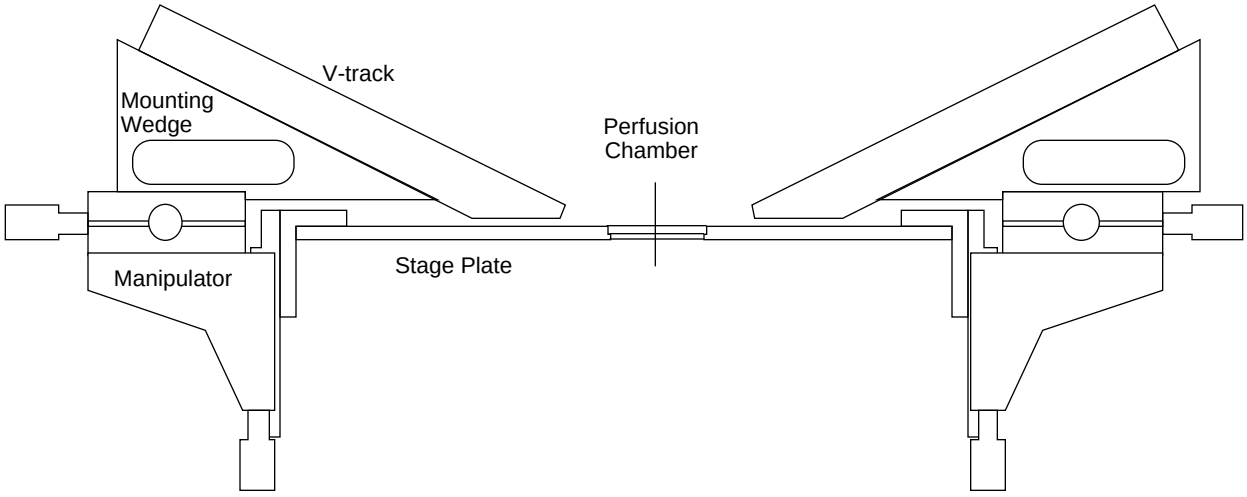

Figure 1: Components of the Vestavia Scientific microperfusion system

I. OVERVIEW

The Vestavia Scientific microperfusion system is the only complete system available for perfusing isolated kidney tubules, sweat ducts, or similar tubular structures. Its main components are shown in Figure 1:

- V-track
- mounting wedge
- XYZ-manipulator
- stage plate
- perfusion chamber

The system can be adapted to upright as well as inverted microscopes using different stage plates and mounting brackets. Details are given in the text *micromanipulations*.

1. V-track

The V-track is at the center of the microperfusion system. The V-shaped track on top receives the pipette holders, the channel in the middle contains the micrometer drive, and the cutout in the bottom connects the track to the dovetail guide on the mounting wedge. For best stability, the V-track is machined from a single piece of metal. Microperfusion requires two V-tracks, one for perfusion and one for collection. By convention, perfusion is from right to left; however, the system can be easily converted to left-to-right perfusion, simply by exchanging the V-tracks.

2. Mounting wedge

The V-track is attached to the manipulator via a special mounting wedge. The slope of the incline is 50% (ca. 27˚). The dovetail guide for mounting the V-track is adjustable to increase or decrease the mechanical play of the connection. The end position on the guide is set by the head of a screw inside the dovetail cutout of the V-track.

3. Manipulator

The manipulator is a 3-stage mechanical translator that is directly attached to the stage plate in a hanging fashion. Alternatively, the manipulator can be mounted to the microscope stage itself. The manipulator comes in a left-handed and right-handed version.

The manipulator can be fitted with different drive spindles, including a joy-stick operated motor drive. However, the basic drive is more than sufficient for most applications. Travel in all 3 dimensions is limited to 13 mm (0.5 inch).

4. Stage plate

The stage plate is mounted like a bridge between the two manipulators. Different stage plates and mounting brackets are available to adapt the system to a variety of microscopes. The stage plate contains a u-shaped cutout for inserting the perfusion chamber (the chamber is described further below).

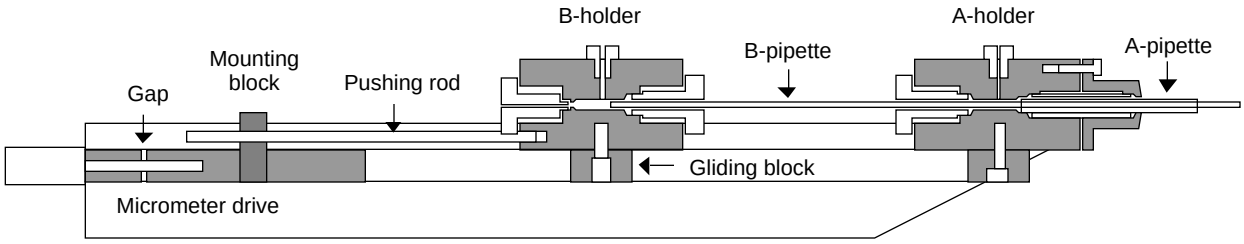

Figure 2: Pipette assembly (perfusion side)

II. SETTING-UP THE SYSTEM

The stage plate is simply placed on the microscope stage and lined up with the optical axis of the instrument. On microscopes with fixed stages, the plate must be connected to the object drive. On microscopes with moving stages, the stage plate can be screwed on using special mounting clamps.

After inserting the drive spindles and adjusting all stages of the manipulator to their middle position, the V-tracks are slipped onto the dovetail guide. Ideally, the tips of the A- and C-pipette should meet in the middle of the u-shaped cutout for the perfusion chamber. Before the chamber can be inserted, the tracks have to be pulled back or the manipulator raised slightly to prevent breaking-off the pipettes.

III. PIPETTE ASSEMBLY

1. Pipettes

The holders are designed for glass pipettes of the following dimensions (in inch):

	o.d.	*i.d.*
A-pipette, C-pipette	.084	.064
B-pipette	.047	.040
Exchange, collection pipette	.020	.014

Suggested length of the A- and C-pipette is 45-50 mm. The B-pipette should be 65 mm longer than the A-pipette; the exchange pipette exceeds the B-pipette by about 20 mm.

2. Setup perfusion side

Slip the B-holder on the V-track. Make sure that the pushing rod runs freely through the opening of the mounting block. Then, mount the A-holder and secure it by tightening the gliding block. The A-holder should be positioned at the edge of the track, exactly as shown in Figure 2.

Release the pressure on all o-rings by loosening the seals and aligning screws. The blunt ends of the A- and B-pipettes must be fire-polished to prevent scratching of o-rings during loading of the pipette.

3. Loading of pipettes

Advance B-pipette filled with perfusion medium backwards through the A-holder into the B-holder until it comes to a complete stop. Back-up slightly so that the end of the pipette is not occluded.

Tighten B-seals until the o-ring is visibly pressed against the holder (area between o-ring and acrylic glass turns black). The seal in the front of the B-holder should be tighter than the seal in the back of the A-holder so that the B-pipette moves together with the B-holder.

The back of the exchange (E-) pipette is glued into the 23 mm long 20 gauge stainless steel tubing. Advance E-pipette (filled with perfusion medium) through the back of the B-holder into the B-pipette until the tip of the E-pipette is close to the tip of the B-pipette. At this time, the steel tubing should be inside the E-seal. Tighten seal gently and rinse pipettes with medium to remove all air bubbles.

Pull B-holder back until the tip of the B-pipette is located in the center of the A-holder, directly above the screw of the gliding block. In this way the tip of the B-pipette is protected during insertion of the A-pipette.

Insert A-pipette into A-holder until it comes to a stop. Tighten all three aligning screws so that the A-pipette is held securely and the gap between front piece and main body of the A-holder is about equally wide on all sides.

Advance B-holder until the tip of B-pipette

is in its desired position within the A-pipette. A *minimum spacing* between A- and B-holder of 13 mm (0.5 inch) must be maintained.

Tighten mounting screw of the micrometer drive. Make sure that B-holder moves freely with the drive and that the drive provides the necessary range of travel. The gap between the stationary and moving part of the drive should be at least 1 mm wide.

4. Pipette alignment

Attach short pieces of PE tubing to all 3 ports of the perfusion side. In this way, connections with the perfusion system can be made without touching the holders again and, therefore, risking the alignment.

Centering of the A- and B-pipette is achieved by adjusting the 3 aligning screws in front of the A-holder. The alignment is facilitated by the stage for pipette alignment, allowing confocal inspection of the pipettes under an stereo microscope (Figure 3).

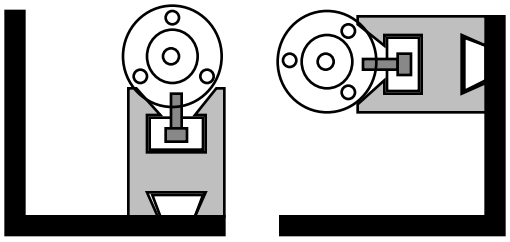

Figure 3: Pipette alignment. *Left,* position for horizontal alignment; *right*, position for vertical alignment.

First, align the A-pipette in the horizontal plane by using one or both of the two lower aligning screws. Then, flip stage by 90˚ and adjust the A-pipette in the vertical plane by using only the upper aligning screw (turning of the upper screw will leave the horizontal alignment intact).

Third, advance the tip of the B-pipette into the tip of the A-pipette using the micrometer drive. The B-pipette should move freely, without bending or hesitation.

Repeat all three steps if the alignment is not satisfactory. After optimal alignment has been achieved, the V-track is transferred to the perfusion rig, slipped onto the wedge, and the remaining tubing connections can be made.

5. Setup collection side

The V-track on the collection side can be loaded in three ways: (1) with only a C-pipette holder, (2) with a C-holder *and* the micrometer drive for collection of fluid samples, or (3) identical to the perfusion side for perfusing in both directions.

C- and A-holder are largely identical. The seal in the back of the C-holder is plugged up when not used for fluid collections. Mounting of the holder and insertion of the C-pipette are identical to the procedures described for the A-holder.

The collection pipette is inserted by hand through the back of the C-holder and, then, fastened to the mounting block of the micrometer drive. The B-seal can be fitted with a thicker o-ring to provide a better seal on thin collection pipettes. Pipettes with o.d. of <0.7 mm must to be glued into a 100 mm long, 20 gauge stainless steel needle. The collection pipette is centered inside the C-pipette using the aligning screws in front of the holder. Axial movements are obtained by the microdrive.

V. MICROMETER DRIVE

The micrometer drive is located inside the channel of the V-track. It is held in place by a set screw which can be inserted into the right or left side of the track for convenient access from the front. The back of the drive must be flush with the back of the V-track.

1. Adjusting the pushing rod

The drives contain blocks for mounting the pushing rod and collection pipette, respectively. Bad alignment of the pushing rod may cause the B-holder to ride unevenly on the V-track. This can be tested by tapping lightly with your finger on top of the B-holder, in front and in the back of the tubing connector. Any difference in feel or sound of the taps indicates that the B-holder is not riding squarely.

To adjust the height of the mounting block, the set screw inside the microdrive has to be loosened. As the screw is accessible only from the front, the drive has to be disassembled and turned around. Adjust the height so that the pushing rod runs parallel to the upper edge of the V-track. Then, tighten the screw and reassemble the drive. The collec-

tion side can be adjusted in the same way.

The pushing rod should slip straight into the mounting block, without hitting the walls. If this is not the case, straighten out the rod by pressing it into the opposite direction. Care must be taken not to bend the rod itself. The adjustment should take place in the connection with the holder. Application of moderate heat (< 60 ˚C) facilitates the adjustment and avoids cracking of the holder.

A sensitive indicator of a good alignment is that the B-pipette does not step sideways (viewed at 50x magnification) when moving the B-pipette forwards and backwards. Sidestepping during a change in direction may also be caused by worn o-rings or excessive mechanical play of the drive. As a last resort, the spring tension of the gliding block may be increased. The tension should be as low as possible, but high enough to provide a firm ride on the track.

New system shipped after July 1998 have a flexible PVC pushing rod which generally does not require any alignment, except for adjusting the height of the mounting block.

2. Operation of the microdrive

The drive must be capable of pushing the B-holder forward by at least 3 mm without any hesitation, i.e. from the starting position indicated in Figure 2 the gap between moving and stationary part should open up smoothly to about 4 mm before stopping and slipping may set in. If the free travel is less, reduce tension of the gliding block or replace the beryllium copper compression spring inside the microdrive. A dry lubricant may be used to reduce the friction between the B-holder and V-track. Maximum travel is about 5 mm.

VI. PERFUSION SYSTEM

1. Perfusion Chamber

The chamber is available in Delrin™ and, if resistance to organic solvents is desired, in Tefzel™. As an option, it can be fitted with a built-in heating coil. To prevent pick-up of noise in electrical recordings, the coil should be powered by a low-noise DC power supply or a battery.

Maximal power rating of the coil is:

- 5 Watt, peak load (< 5 sec)
- 2 Watt, continuous load (chamber at least filled by half, < 40°C).

The electrical resistance is about 10 Ω (exact values will vary). The coil is embedded a special heat-conducting cement.

The chamber rests in a U-shaped cutout of the stage plate for rapid insertion and removal. It is held in place by two spring-loaded clips. Strain relief blocks for making tubing connections and a bath electrode for electrical recordings are available as accessories.

2. Fluid flow system

Connections are made by 0.86 mm i.d. polyethylene tubing sections (Intramedic PE-90) which are slipped over 20 gauge stainless steel needles. For switching the flow, 3-way miniature valves with 20 gauge needle fittings are recommended (Hamilton HV series). All connections must be air-tight.

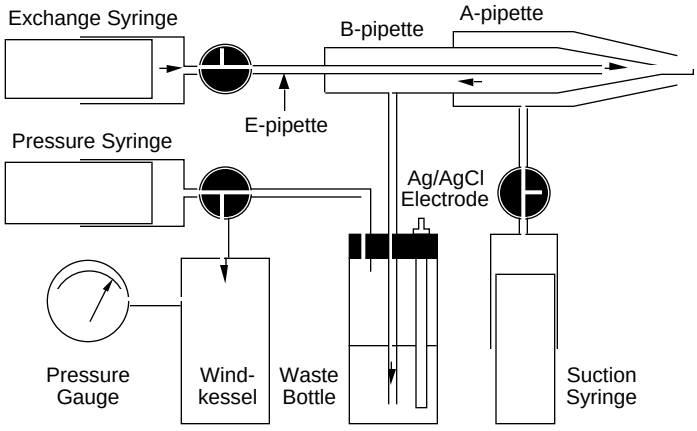

Figure 4: Fluid flow system (perfusion side only). For details see text.

Figure 4 depicts the fluid flow system on the perfusion side. The upper ports on the A- and B-holders are connected to the suction syringe and waste bottle, respectively. The port in the back of the B-holder is connected to the exchange syringe.

The arrows in Figure 4 indicate the fluid movement during a solution exchange. While perfusing, the solution actually flows back from the waste bottle, driven by the pressure in the windkessel. However, the flow rate is very slow, provided that the system is tight.

The pressure in the windkessel (air tank) is adjusted by the pressure syringe and should be monitored by a pressure gauge. To keep the

pressure stable (<10%/hr), the volume of the windkessel should be at least 1 liter.

3. Electrodes

Three different potential recording electrodes are available: (1) a bath electrode for the perfusion chamber, (2) a waste bottle electrode, and (3) a B-seal electrode which fits into the back of the C-holder. All three types feature low-maintenance Ag/AgCl pellet electrodes (In Vivo Metric, E206) and male gold connectors (AMP 60599-3, type A). Figure 5 shows a cross-section through the bath electrode.

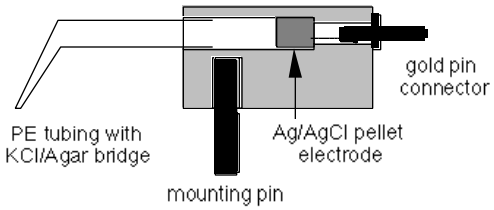

Figure 5: Bath electrode, finished with built-in Ag/AgCl pellet electrode, gold connector, and mounting pin. The tubing has an o.d. of 2.08 mm (Intramedic PE-205).

Saturated KCl/Agar or similar salt bridges should be used to connect the electrodes with the bath and luminal side. When using the waste bottle for electrical recording, the level of fluid in the bottle must be high enough to reach the opening of the tubing leading to the B-pipette and the Ag/AgCl electrode (Figure 4). Moreover, pre-filling of the waste bottle with saturated KCl is required to minimize fluid junction potentials.

VI. CLEANING/MAINTENANCE

Lucite™: Use a mild detergent solution for cleaning. Parts should be soaked for 10 min and rinsed thoroughly with deionized water. Use special cleaning brush to remove glass splinters or dirt particles trapped inside the holders. Do *not* expose to organic solvents.

PVC: Clean with alcohol or mild detergent solution. Do not expose to strong organic solvents, such as acetone.

Anodized aluminum: Keep exposure to salt or salt solutions to a minimum. Clean with acetone or any other organic solvent.

Brass and stainless steel: Keep exposure to salt or salt solutions to a minimum. Clean with acetone or any other organic solvent. Surfaces should be covered with rust-inhibiting machine oil. Stains can be removed with a metal polish (e.g. Brasso™).

Tefzel™ and Delrin™ parts do not require special care.

Pressure on the o-rings should be released when the system is not in use. The o-rings must be replaced at least once every year. The compression spring inside the micrometer drive should be replaced only when showing signs of fatigue (i.e., reduced tension).

The drives spindles must be kept dust-free and should not be exposed to salt solutions. A protective cover is available as accessory. Keep spindles lubricated with rust-inhibiting machine oil or silicone grease.

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