OPERATIONS MANUAL

MULTICHANNEL VOLTAGE-CURRENT CLAMPS

MODEL VCC MC2 REVISION A
MODEL VCC MC6 REVISION B
MODEL VCC MC8 REVISION A

SERIAL NO. _____

PHYSIOLOGIC INSTRUMENTS, INC.

5875 Tyrone Road
Reno NV 89502
Tel: (858) 451-8845
Email: office@piusa.net
Web: http://www.physiologicinstruments.com

Table of Contents

PHYSIOLOGIC INSTRUMENTS

MULTICHANNEL VOLTAGE-CURRENT CLAMP
MODEL VCC MCx

Instrument Description

The model VCC MCx[1] is a multi-channel voltage/current clamp designed to be a flexible tool to enable study of ion transport mechanisms by *in vitro* epithelial tissues. The VCC MCx provides the capability to control either the voltage or the current across epithelial tissues, thereby enabling one to measure electrophysiological responses to drugs, hormones, and/or ion substitutions as well as to perform measurements of ionic flux. The VCC MCx is suitable for use with both "leaky" and "tight" epithelia, tissue samples or cell culture, large tissue area or very small. The model VCC MCx offers features such as:

1) A built-in pulse generator for monitoring of the tissue conductance.
2) Up to two, six, or eight (depending on the model) independently controlled voltage/current clamp channels.
3) Digital readout of the transepithelial voltage or current.
4) Compensation for electrode asymmetry potentials over user selectable ranges of ±10 mV or ±100 mV.
5) Automatic compensation for voltage errors due to the resistance of the fluid in series with the epithelium - fluid resistance compensation.
6) The ability to clamp either the voltage or current to any dc level in the range ±100 mV or □A.
7) A Master Control switch that permits "one-touch" control of all clamp units.
8) Recorder outputs for continuous monitoring of the transepithelial voltage and current.
9) A pulse generator output providing +, - or bipolar pulses.
10) An external command signal input to allow an arbitrary waveform to be clamped.
11) A remote-control interface jack to allow the VCC MCx to be controlled directly by computer using the ACQUIRE & ANALYZE© data acquisition and analysis system or other such remote interface.

[1] Throughout this document we will refer to the family of MCx units as VCC-MCx, where “x” is the number of channels. We have three models, the MC2, the MC6, and the MC8. Most of the details are identical, with a few notable exceptions. Where there are specific characteristics to each model, they will be identified as such.

Installation

Before plugging in the instrument……

Setting the Mains Voltage

Electrical mains supply differs throughout the world but has reached some standardization. The VCC MCx instruments are designed to operate on many of these standards. In the United States the standard is 120V at 60Hz (less common, but sometimes available in labs is 240V at 60Hz). Europe typically uses 230V at 50Hz. Japan uses 100V at either 50 or 60Hz. Your country may be different, but it will supply a voltage somewhere in the ranges 100-127V and 220-240V at 50 or 60 Hz. The VCC MCx has been designed for these supply ranges but ***does*** require that the proper mains voltage settings in the power entry module be selected. To verify the mains power selection, look at the reading in the small widow on the AC POWER INPUT MODULE on the rear of the instrument. To determine the correct setting for AC supply, use the table below.

Instrument	**VCC MC2**		**VCC MC6 / 8**	
AC Voltage Range	100-120V	220-240V	100-120V	220-240V
Power Input Setting	115	230	100 or 120	220 or 240

To change the setting on the power input module, insert the blade of a small screwdriver into the slot at the top of the module and apply leverage to open the module. On the VCC MC2 remove and rotate the fuse holder being careful that the fuse remains in place. On the VCC MC6/8 remove the small drum at the top of the module and reinsert with the correct voltage displayed.

IMPORTANT: If the power input selector is not correctly set to the mains voltage, *severe damage* to the instrument can result. Make sure you check with your building and/or lab safety manager to verify the mains voltage before connecting this equipment.

Fuse Selection

The instruments are typically shipped with a 0.5A, 250V fast acting fuse installed. For Europe this fuse is replaced with a 0.315A, 250V fast acting fuse that is designed to the IEC 60127-2-1 standard. For CE certified instruments being used in Europe this latter fuse style must be used in order that the CE certification be in effect.

General Setup

Placement of the VCC MCx instrument with respect to the chamber system is not critical. However, common sense suggests that where possible the instrument should be set above and behind the chamber system to avoid damage by saline solutions. This also applies to the DM_MC6 input modules. A 36” electrode lead cable is supplied with our EasyMount chamber systems to facilitate this placement of the signal input module.

Description of Controls

The VCC MCx is divided physically and functionally into sections. The upper part of the instrument contains a bank of 2, 6, or 8 (MC2, MC6, MC8) independent voltage-current clamps while the lower part (center panel on the VCC MC2) contains the pulse generator and dc clamp level controls, the Master Control, and the power switch. Controls for the independent voltage-current clamp sections will be described first. Switch positions are indicated by light emitting diode (LED) indicators.

Figure 1: Clamp Channel

I. Clamping Circuitry

Offset

The Offset is used to compensate for voltage differences between the measuring electrodes. The range is ±10 mV or ±100 mV, set by an internal jumper. Adjustment is by means of a 10-turn calibrated potentiometer knob after pressing the OFFSET switch to a + or - polarity. For electrodes that are well matched, a range of ±10 mV is sufficient. For electrodes that are not well matched, but are stable, the ±100 mV range should be selected (see Table II). Electrodes that are not stable with time should be reconditioned or discarded.

Fluid Resistance Compensation

The Fluid Resistance Compensation (FRC) circuitry is used to correct for non-tissue related resistance in the chamber. If this is not done properly, measurements of tissue resistance will not be accurate. The FRC range is typically 0-100 Ω but is also user selectable by internal jumpers (see Table II). FRC is adjusted by pressing pushbutton switch labeled PUSH TO ADJ to pass a test current then turning the 10-turn calibrated potentiometer knob to nullify the voltage change. To make the adjustment the FUNCTION switch (see below) must be in the OPEN position.

Function

The FUNCTION switch determines whether: 1) the inputs of the voltage sensing amplifiers are to be internally grounded (ZERO, no lights), 2) the feedback circuitry is open circuited (OPEN, green light) so that the epithelium is in the "open circuit" condition, or 3) the feedback loop is completed (CLAMP, red light) so that the tissue is either voltage clamped or current clamped at the level given by the command signal (see below).

Mode

Current / Voltage

Selects whether the transepithelial current (red) or the transepithelial voltage (green) is to be clamped when the FUNCTION switch is set to CLAMP.

Remote

Determines whether the selection of clamp FUNCTION, CURRENT/VOLTAGE MODE, and the clamp level are to be controlled using the front panel controls (local mode) or by external device such as a computer (REMOTE - amber LED on).

Meter

Selects either the transepithelial current (red) or voltage (green) to be displayed on the panel meter. Displayed voltage is V_2-V_1. Transepithelial current displays as positive for cation flow across the tissue from side 2 to side 1. For example, for the toad urinary bladder that actively absorbs the cation sodium, the transepithelial voltage is typically negative in the lumen with respect to the blood side (e.g., -50 mV). If V_1 and I_1 are connected to the blood side of the epithelium, then the displayed spontaneous transepithelial voltage V_2-V_1 will be 50.0 mV while the short-circuit current will be positive. The meter may be read directly in mV or □A over the range ±199.9 mV or □A. Note: the gain of the current measuring amplifier may be altered using internal jumpers (see Internal Jumper Selection below).

II. Pulse Generator & DC Clamp Level

Pulse (On – Off)

Turns pulse on (red) or off as a command signal to the clamp circuitry and to the PULSE OUTPUT connector on the rear panel.

Polarity

Selects polarity of unipolar pulses designed so that + (red) and - (green) yield positive and negative step changes in the clamped parameter (voltage or current), respectively. When both LED's are on, bipolar pulsing is selected (the pulse polarity and the order of pulse (positive or negative first) in bipolar mode is user selectable by internal jumper).

Reset

Resets the period timer to zero and elicits a pulse.

Single Pulse

Injects a single pulse without interrupting the period timer.

Period

Allows digital selection of the interpulse interval in seconds. The range is 0-99.9 sec.

Duration

Allows digital selection of the duration of the pulse in seconds. The range is 0-9.99 sec. Restriction: the duration may not exceed one-half the period or timing errors will result.

Amplitude

Allows selection of pulse amplitude in steps of 1 mV (or □A) over the range 0-10 mV (□A) (red LED) or in steps of 10 mV (□A) between 0 and 100 mV (□A) (green LED) when used with the clamp circuitry. Pulse amplitude at the PULSE OUTPUT connector will be 10x this value (mV only).

DC Clamp Level

Determines the D.C. level at which the voltage or current will be clamped. The polarity is switch selectable (+ is red, – is green) while level is set by means of a 10-turn calibrated potentiometer. Range: ±100 mV or □A.

Figure 2: Lower Front Panel VCC MC6

III. Master Control

This is included with the MC6 and the MC8, and optional with the MC2. This section is used to control the clamp FUNCTION, MODE and METER switches of all individual clamp channels simultaneously. Master Control is activated by pressing the Master Control switch with ON being indicated by an amber LED. Upon entering the MASTER CONTROL mode, each clamp channel is set to the following initial conditions: FUNCTION = Zero (both function LED's off); MODE = Local, Voltage; and METER = Voltage. Pressing any of the control switches in the Master Control section will then cause each installed clamp channel to follow the command (e.g., pressing Function once will cause each channel to change to OPEN). While using the Master Control feature, controls on the individual clamp channels will be unresponsive; however, their LED's will display their current status. To exit the Master Control and return control to the individual units, press the Master Control switch to extinguish the amber LED. Unlike entry into Master Control, which must establish initial conditions so that all clamps have the same status, exiting this operation will leave the units in their current state.

Figure 3b Optional Current Gain and Fluid Resistance Controls

Option Gain Control

The option for Gain and Fluid Resistance (not currently available on VCC MC2) adds the capability of setting the current gain and fluid resistance compensation ranges from a pair of rotary switches located just below the Master Control section rather than by moving internal jumpers. The left switch has 3 positions which can set the gain for current measurement so that the output is 1, 10 or 100 mV/μA. The right-hand switch sets the fluid resistance range in Ohms to 10, 20, 50 or 100 times the current gain. For example, if the current gain is 10 and fluid resistance is set to 20, the fluid resistance compensation range is from 0 to 200 Ohms. If the settings for current gain and fluid resistance are 100 and 50 respectively, the fluid resistance range is from 0 to 5000 Ohms.

Please note that when you change the current gain on the instrument you must set the instrument gain in the A&A program to the same value.

IV. Power

Turns device ON or OFF.

V. Rear Panel Input / Output Connections

All I/O connections are via the rear panel.

Current

BNC output connector. Allows continuous monitoring of the transepithelial current. Output gain is factory set to 10mV/μA but is jumper selectable (see Table 1). Current output is positive for current flow (direction of cation movement) from side 2 to side 1.

Voltage

BNC output connector. Allows monitoring of the transepithelial voltage (V_2-V_1). Output is at x10 gain - i.e., 10mV/mV.

Input

Connection of the voltage and current electrodes inputs from the chamber to the main chassis can be made for each channel individually or for six channels altogether.

Single Chanel Input Module

A 6-pin modular plug. One standard modular input connector is provided for each clamp channel for connection to a single channel electrode interface box. The interface box provides pin tip jacks for connection to electrodes.

Electrode Panel Input

For the MC6 and MC8 only. A 34-pin connector. An electrode input panel containing 24 pin tip jacks is provided for connecting electrodes for up to six chambers. This panel is designed to mount on 0.5" diameter rods and is compatible with the Navicyte vertical chamber system from Harvard Apparatus, Inc.

One type of input interface is supplied as specified at time of order.

External Input

Allows any external waveform to be clamped. The signal is reduced 10-fold so that a 100 mV input signal will cause the tissue to be clamped at 10 mV or □A depending on the clamp MODE.

Pulse Output

Allows monitoring or use of the pulse generator output when the PULSE ON/OFF switch is in the ON position. Output is at x10 gain. (Note: By connecting the PULSE OUTPUT to the EXT. INPUT the range of the PULSE AMPLITUDE is exactly doubled - e.g. ±200 mV in 20 mV increments.)

Remote Interface

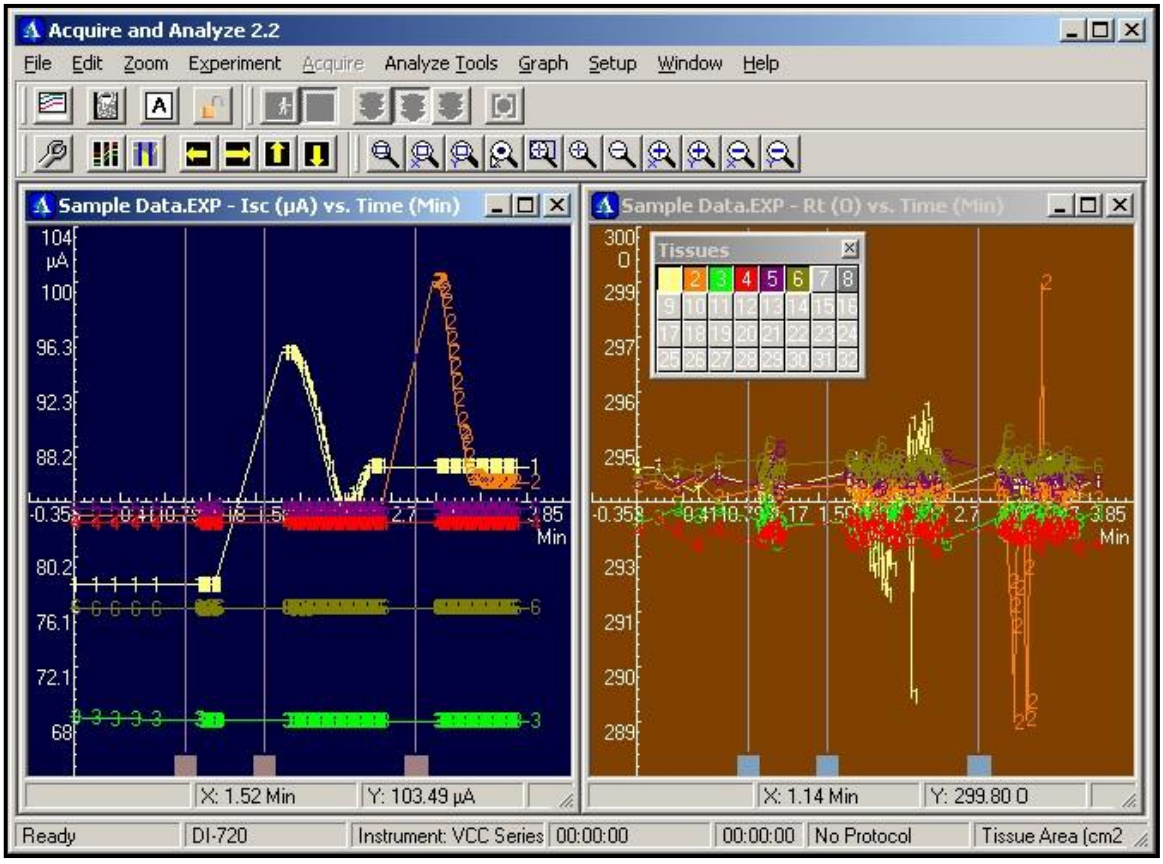
The remote interface is accessible via a 25-pin D-sub connector on rear panel. Provides control lines to permit setting FUNCTION and MODE switches by external device such as a computer and output lines for recording voltage and current for each clamp channel. Interface is compatible with Acquire & Analyze, a Windows□[2] based data acquisition and analysis system written specifically for studies of epithelial ion transport. Acquire & Analyze[3] is available from Physiologic Instruments, 5875 Tyrone Road, Reno, NV, 89502; (858) 4518845.

[2] Windows is a trademark of Microsoft, Inc.

[3] Acquire & Analyze is copyright 2006 by Physiologic Instruments, Inc.

Acquire & Analyze (A&A)

A&A is a comprehensive data acquisition and analysis package designed specifically to study in vitro epithelia controlled by VCC600, VCC MC2, VCC MC6 or VCC MC8 voltage/current clamps. Version 2.2 (see screenshot below) provides many additional acquisition features including experiment protocol design with alarms, use of manipulations libraries, ability to turn data collection on and off for individual tissues, and ability to generate current/voltage relations (optional). Analysis features include initial rate calculations (slope), area under curve (e.g., charge transfer), data averages with output to Excel, tissue area correction, multiple graph windows, user-definable colors, symbols for identifying tissue data, and zoom features.



General Operating Instructions

Startup

Before powering up the instrument please verify the main voltage setting (see Setting the Mains Voltage). Turn the POWER switch to ON. Allow the instrument to warm up for at least 2 minutes. Upon power up the following conditions will be established: FUNCTION switch set to ZERO (both LED's off); the MODE switches to LOCAL (REM LED off), VOLTAGE (green);

METER set to display voltage (green); OFFSET set to off (both LED's off); PULSE GENERATOR set to off, bipolar, and x1; DC CLAMP LEVEL set to off; and MASTER CONTROL off. All calibrated potentiometers should be set to 0.0 (i.e., turned fully counterclockwise).

Connect the voltage sensing and current passing electrodes to the instrument either by way of the SINGLE CHANNEL INPUT MODULES (DM_MC6 -see page 22) or via the ELECTRODE PANEL (EP6, for MC6 only).

Typical Experiment

Fill an "Ussing" type chamber with physiological saline and connect appropriate electrodes to inputs V_1 and V_2 for measuring of the transepithelial voltage. Connect current passing electrodes to I_1 and I_2 ensuring that V_1 and I_1 are connected to the same side of the epithelium. The digital panel meter should read zero when the METER switch is in either the VOLTAGE or CURRENT position. Switch the FUNCTION switch to OPEN (green) and the METER switch to VOLTAGE (green).

Figure 4: EasyMount 6-Channel Ussing Chamber System

The voltage (V_2 - V_1) is the asymmetry voltage between the voltage measuring electrodes and is the sum of asymmetry in the electrodes themselves and all liquid junction potentials in the voltage measuring circuit. To compensate for these, place the OFFSET switch in either the plus (red) or minus (green) position and adjust the OFFSET potentiometer so that the voltage reads 0.0 on the panel meter. If the reading on the meter is not stable, is off scale (i.e., > ±199.9 mV), or cannot be compensated, the problem usually involves an air bubble or discontinuity in one of the voltage sensing limbs of the circuit (e.g., agar bridge or electrode). When the electrode OFFSET is properly adjusted place the METER switch on CURRENT and press and hold the FLUID RESISTANCE COMPENSATION test button labeled: PUSH TO ADJUST.

Verify that a current of approximately 60-68 □A is registered on the meter.

NOTE: Values less than this indicate that the resistance of the current passing bridges or electrodes is excessive. While this in itself will not prevent the clamp from working, it does limit the amount of current that can be passed and is poor experimental design in that it increases errors due to non-ideal rejection of the common mode voltage resulting from current flow between the chamber and the I_1 input. It is recommended, therefore, that the resistance of the current passing bridges be kept low by making them as short as possible and/or by increasing their cross-sectional area. For reference, typical electrode resistances in the EasyMount Ussing chambers are 1-2 kΩ.

While still passing current (by continuing to press the PUSH TO ADJUST button), press the METER switch until VOLTAGE is displayed and then adjust the FLUID RES COMP

potentiometer so that V_2-V_1 on the panel meter again reads 0.0. At this point the value of the resistance due to the fluid in series with the tissue may be read from the calibrated dial and will be compensated for automatically at any current - i.e., the actual voltage across the epithelium will be determined and will appear on the digital panel meter and at the VOLTAGE OUTPUT BNC connector (at x10 gain). In the event that a current cannot be passed when the test button is pushed, the problem usually involves an air bubble in one of the current passing agar bridges.

Now place the FUNCTION switch back on ZERO (both LED's off) and mount a tissue in the chamber being careful not to change the distance between the tips of the voltage sensing electrodes. Place the FUNCTION switch on OPEN and the METER switch on VOLTAGE to read the open circuit voltage generated by the tissue (already corrected for electrode asymmetry potentials). Place the FUNCTION switch on CLAMP with the MODE switch on VOLTAGE and verify that (V_2-V_1) now reads 0.0 - i.e., the tissue is short-circuited. Place the METER switch to CURRENT to read the short circuit current, Isc. The transepithelial voltage may now be clamped to a user specified voltage using the CLAMP VOLTAGE or CURRENT and/or PULSE GENERATOR circuitry.

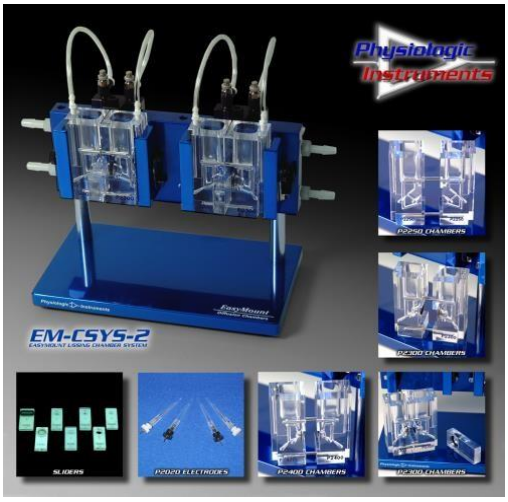


Figure 5: EasyMount Ussing Chambers

The MASTER CONTROL section will permit you change clamp function, clamp modes and meter settings for all clamp channels. Because all installed clamp channels will follow commands from the MASTER CONTROL, using this feature requires that each installed channel must either be connected to a chamber via electrodes or be connected to a dummy load. For instruments shipped with the DM_MC6 input stage, a dummy test membrane is built into the headstage so that the slide switch simply needs to be pushed to "Test" instead of "Operate" (see instructions DM_MC6 use - p. 16). For instruments shipped with the 6-channel electrode input panel, a separate modular plug is provided for each clamp channel which serves as a dummy load and should be connected via the SINGLE CHANNEL INPUT MODULE connector for each unused clamp channel. The reason for this is that if no load is connected to an individual clamp channel, then placing that channel in voltage clamp mode will cause the output of the clamp amplifier to saturate and may influence the output of other clamp channels (via the shared power supply) resulting in erroneous measurements. This is circumvented either by not using MASTER CONTROL when all channels are not in use, or by using the dummy loads on unused channels.

Remote Control

When the MODE switch is placed on REMOTE the front panel FUNCTION (OPEN or CLAMP) and MODE (clamp I or V) controls are disabled. All other controls are unaffected. Control of

these disabled features must be supplied via the REMOTE interface on the rear panel. Details of this feature are given in Table 1 on Page 13.

Remote Interface Connections

On the rear panel is a 25-pin female D sub-miniature connector by which interface may be made to an external controller such as a computer or a simple battery and switch. A compatible interface cable is provided with the ACQUIRE & ANALYZE hardware/software data acquisition system.

The pin connections are shown in Table I.

Table I. Remote Interface Connection for VCC MC2

PIN#	DESCRIPTION
	Note: Vm OUT and Im Out are for each channel/DB9 connector
1	Selects CURRENT or VOLTAGE MODE: this must be a TTL compatible logic signal. Logic high (+3.5-5 V) selects CURRENT, logic low (0-0.8 V) selects VOLTAGE.
2	Analog ground (reference signal for A/D measurements).
3	V_m OUT - Transepithelial voltage (V_2-V_1) at 10 mV/mV (same signal as at the VOLTAGE BNC connector) from clamp channel.
4	Analog signal input low: this signal may be the analog ground of the D/A converter that typically should be isolated from the system ground.
5	Not connected.
6	Selects FUNCTION - OPEN or CLAMP; logic high (+3.5-5 V) selects CLAMP; logic low (0-0.8 V) selects OPEN.
7	Analog signal input high: this signal replaces the CLAMP VOLTAGE or CURRENT signal and may be the output of a D/A converter, etc.
8	I_m OUT -. Transepithelial current (same as at the CURRENT BNC connector) from clamp channel.
9	Chassis ground

Table II. Remote Interface Connection for VCC MC6 / 8

PIN#	DESCRIPTION
1	Selects FUNCTION - OPEN or CLAMP; logic high (+3.5-5 V) selects CLAMP; logic low (0-0.8 V) selects OPEN.
2	Selects CURRENT or VOLTAGE MODE; this must be a TTL compatible logic signal. Logic high (+3.5-5 V) selects CURRENT, logic low (0-0.8 V) selects VOLTAGE.
3	Circuit or signal ground. (Note: This should not be connected to the signal ground on the computer or unwanted noise may result.)
4	Analog signal input low: this signal may be the analog ground of the D/A converter that typically should be isolated from the system ground.
5	Analog signal input high: this signal replaces the CLAMP VOLTAGE or CURRENT signal and may be the output of a D/A converter, etc.
6	Analog ground (reference signal for A/D measurements).
7	I_m OUT - Channel 1. Transepithelial current (same as at the CURRENT BNC connector) from clamp channel 1.
8	V_m OUT - Channel 1. Transepithelial voltage (V_2-V_1) at 10 mV/mV (same signal as at the VOLTAGE BNC connector) from clamp channel 1.
9	I_m OUT - Channel 2.
10	V_m OUT - Channel 2.
11	I_m OUT - Channel 3.
12	V_m OUT - Channel 3.
13	I_m OUT - Channel 4.
14	Shield or Chassis ground.
15	Not connected.
16	Not connected.
17	V_m OUT - Channel 8.* * VCC MC8 only
18	I_m OUT - Channel 8.*
19	V_m OUT - Channel 7.*
20	I_m OUT - Channel 7.*
21 -	V_m OUT - Channel 6.
22 -	I_m OUT - Channel 6.
23 -	V_m OUT - Channel 5.
24 -	I_m OUT - Channel 5.
25 -	V_m OUT - Channel 4.

Internal Jumper Configurations

The VCC MCx user may select optional instrument setup parameters via seven internal jumpers (JP) - six on each clamp channel and one on the main board at the bottom of the instrument. The purpose and setting options for each jumper are described below. Factory default settings are indicated by the "*" marking (See Table II).

Note: Positions A,B,C, etc. are from left to right. See Figure x for jumper location. The position of J1 on the pulse main board is:
#VCC MC2 – J1 located at rear top of center pcb. Access from top of instrument.
##VCC MC6 / 8 - on Main circuit board inside the unit to left of U34 – access by removing left side panel.

Table III. Internal Jumper Settings for Clamp Channel

JUMPER	LOCATION	FUNCTION or SETTING
JP1	(to right of U23)	Sets fluid resistance compensation range. The range is given by the product of the current amplification determined by JP2 and the factor selected below: *A - x0.01 B - x0.02 C - x0.05 D - x0.10 For example: JP1-B, JP2-B yields a resistance compensation range of: J1-B J2-B 10 mV/□A * 0.02 = 200 □
JP2	(next to TP1)	Sets the voltage output gain of the current measuring amplifier. A - 1 mV/ □A *B - 10 mV/□A C - 100 mV/□A
JP3	(below trim pots)	Sets OFFSET adjustment range: *A - ± 10 mV B - ± 100 mV

JP4		Sets the decimal point location for the displayed current only. Proper setting of this jumper depends on the selections made for JP2. A - ± 1999 *B - ± 199.9 C - ± 19.99 D - ± 1.999
JP5		Selects power supply voltage to the clamp amplifier (U18). Settings must be made in pairs - e.g., B & C or A & D. A - -35 V (option HV only) *B - -15 V *C - +15 V D - +35 V (option HV only)
JP6		Determines whether the Pulse and Level command signals are operative in Remote Mode or are disabled. A - enabled (these signal will be added to the Remote command signal). *B - disabled. (Pulses and clamp Level are disabled as command signals when the clamp channel is in Remote Mode).
JP7*	Rev D and later	Changes the amount of current injected when the fluid resistance button is pressed. A – Injected current ~67µA B - Injected current ~15µA Note: *This position is used when the current gain is set to 100 mV/µA –JP2 position C.*
J1	#VCC MC2 ##VCC MC6 / 8	Changes the order in which pulses are generated or reverses the polarity of the pulse indicated on the front panel. *A - normal polarity. Positive going pulse precedes negative pulse. *B - reverse polarity. The polarity of the pulse generator output is reversed from that indicated by the front panel LEDs. In bipolar mode it reverses the order of the sequential pulses.

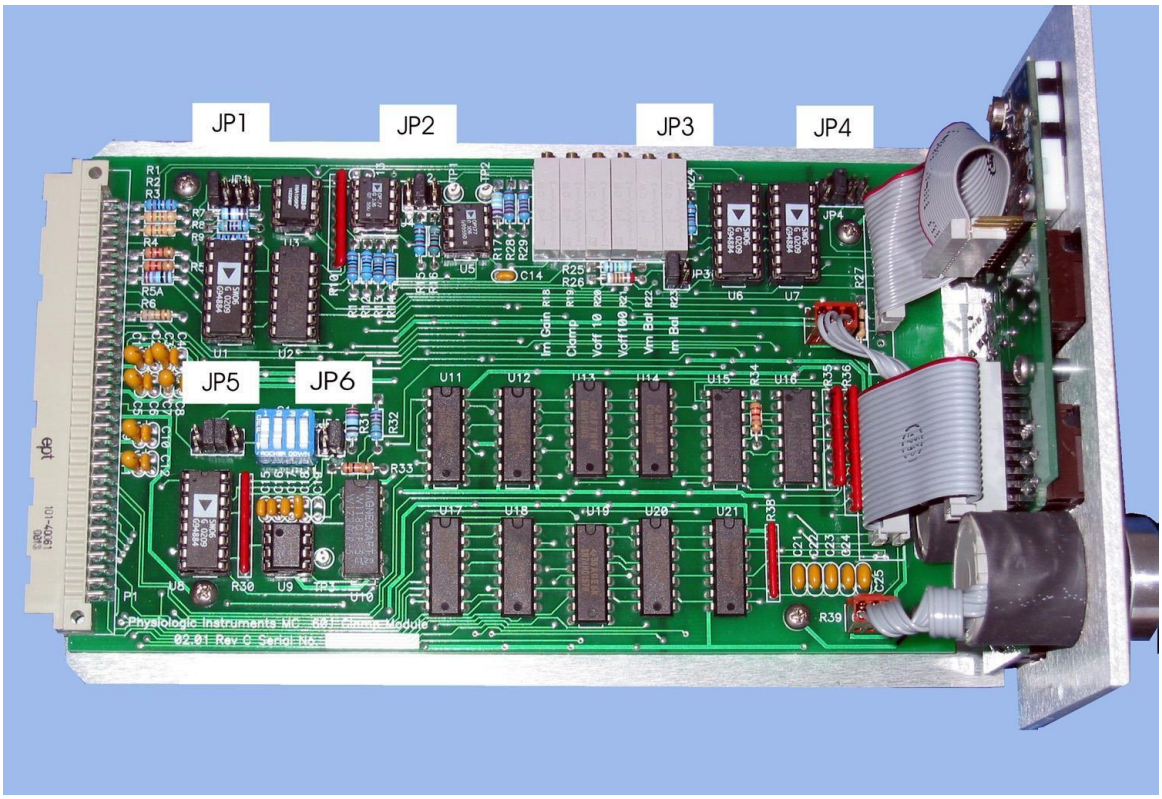


Figure 6: MC_601 Clamp Channel – Jumper Locations

Instrument Calibration

The following is a simplified calibration/balance procedure. For the most part it is all that is needed to quickly "tune-up" the instrument.

Quick Balance Procedure

1. Turn instrument on to generate a power up sequence and establish initial conditions as described above under General Operating Procedures.
2. Remove the top cover of the VCC MCx by removing the 4 screws and lifting the top cover vertically (applying suction via a suction cup or a piece of tape can make removing the cover easier). Do not lose the screws! They are metric (M3) and hard to replace if lost.
3. Unplug the electrode panel input cable from its connection at the rear of the instrument and plug the test dummy membranes shipped with the instrument into the modular input connector of each channel to be balanced.
4. Wait 10 minutes for instrument warm up.
5. Locate the five trim pots on each clamp channel. From front of instrument to back these are: Current balance (Im Bal), Voltage balance (Vm Bal), ±100 mV range Offset calibration (Voff 100), ±10 mV range Offset calibration (Voff 10), Clamp amplifier balance (Clamp),

and Current (Im Gain) calibration. ***ONLY*** Ioff Bal, Voff Bal, and Clamp will be adjusted in this procedure. Be careful not to accidentally adjust the wrong trim pot.

6. Using the Master Control switch set the meter on each channel to read Current (red). Adjust Im Bal on each channel to read 0.0 on the digital panel meter. **Hint:** The polarity sign on the digital meter changes (appears/disappears) right at 0.00 mV so that by slightly adjusting the trimpot screw the polarity sign will toggle on and off resulting in very close balance.
7. Set the meter switch to Voltage (green) and adjust Vm Bal to read 0.0 mV on each channel.
8. Press the Function switch twice to place each channel in voltage clamp mode. Adjust the trimpot labeled Clamp to read 0.0 on the meter.
9. Replace the top cover panel.

General Balance / Calibration Procedure

Equipment Needed

1. Dummy membrane with internal voltage source (e.g., DM_MC6 or DM6).
2. Digital voltmeter (DVM) having sensitivity of 100 □V (0.1 mV).
3. Oscilloscope with vertical input sensitivity of at least 2 mV/div is useful, but not mandatory.

Instrument Calibration

The following describes general calibration procedures for the VCC MCx instruments. Because of different physical layouts, the locations and component identifiers for the VCC MC2 will be given in braces and in a different font; e.g. **{font for VCCMC2}.**

1. Turn the instrument power off and then on to generate a power up sequence and establish initial conditions as described above under General Operating Procedures.
2. **{Skip for VCCMC2}** Remove the side panel from the left side of the instrument to gain access to the trim pots and test points on the main circuit board. This is done by placing both hands over the mounting rail on the left side and firmly grasping the side panel between thumb and forefingers. Press downward and outward to release the panel that is held into slotted channels by rubber gasket strips.
3. Unplug the electrode panel input cable from its connection at the rear of the instrument and plug the small test fixtures (passive membranes) shipped with the instrument into the modular input connector of each channel to be balanced or optionally use an active test dummy such as our DM_MC6 Single Channel Dummy Membrane or DM6 Six Channel Dummy Membrane.
4. Wait 10 minutes for instrument warm up.
5. Locate the 7 trim pots and 4 test points on the main circuit board at the left side of the instrument **{Top of central pcb}.**

Steps 6 & 7 will calibrate the +10 and -10 volt reference sources.

6. Attach the negative input of the DVM to chassis ground and the positive probe to test point TP3 on the main circuit board (second from front). Adjust trim pot R39 **{R24}** (second from front) so the voltage reads +10.000 volts.
7. Move the probe to TP4 and adjust R41 **{R25}** (first from front) to read -10.000 volts.

Steps 8-11 will balance and initially calibrate the pulse generator and the DC Clamp Level outputs.

8. Attach the probe to TP1. Set the Pulse Period to 90.0 sec and the Duration to 4.00 sec. Ensure that the DC Clamp Level is set to Off (no LED's lighted). Set the sensitivity of the DVM to 200 mV range and adjust R29 **{R19}** (first trim pot from rear) to read 0.0 mV on the DVM.
9. Turn the Pulse to ON, set the amplitude to 10, x1. Press and hold the Reset switch to initiate a pulse and lock it in the high state. Adjust R31 **{R20}** so that the DVM reads 100.0 mV. Release the Reset switch and verify the Pulse remains high for 4 seconds and then pulses to -100.0 mV for 4 seconds before returning to zero.
10. Set the DVM sensitivity to 2 V range. Set the Pulse Amplitude Gain to x10 and repeat step 9 while adjusting R33 **{R21}** (third from rear) so that the pulse reads 1000.0 mV. Turn the Pulse to Off (no LED).
11. Set the 10-turn dial for the DC Clamp Level to 60.0 (6 in the dial window). Verify that the DVM still reads 0.0 mV. Press the DC Clamp Level switch to + (red) and adjust R35 **{R22}** (fourth from rear) for an output voltage on TP1 of 600.0 mV. Press the switch to - (green) and verify that the meter now reads -600.0 mV.

Step 12 will balance the Remote interface buffer amplifier.

12. Connect the probe to TP2 and adjust R37 **{R23}** (third trim pot from front) to read 0.0 mV on the DVM.

Steps 13 - 18 describe the calibration of a single clamp channel. Repeat for each channel installed. Review the Quick Balance Procedure above for location and description of the trim pots and test points.

13. Balance the current measuring amplifier: Set the instrument to the startup condition. Place probe on TP2 of the clamp module. Set the meter switch to Current. Adjust Im Bal to read 0.00 mV on the DVM and 0.0 on the meter.
14. Calibrate the current measuring amplifier: Connect a test resistor probe between TP3 on the main circuit board (+10.000 volt reference) and the top most pin on the DIN connector at the back of the clamp module. The test resistor probe should impose a fixed resistor (e.g., 500 KΩ will give a test current of 20 □A) between the reference voltage and the input of the current measuring amplifier. This current can be measured directly by using the DVM as an ammeter between the voltage reference and the resistor or by accurately measuring the

resistance and calculating the current using Ohm's law. Once the test current is established adjust the Im Gain trim pot to display the correct value for the current.

15. Balance the voltage-measuring amplifier: Move the probe to TP1 to monitor the membrane voltage. Set the Meter to Voltage. Adjust Vm Bal so that the meter reads 0.0 mV. This balances the voltage-measuring amplifier.
16. Calibrate the voltage offset for ±10 mV range: Move jumper JP3 (located immediately below the Im Bal trim pot) to position A. Set the Offset 10-turn dial to read 6.0. Press the Offset switch to + (red). Adjust the Voff 10 trim pot to read 6.0 mV on the meter. Press the Offset switch to - (green) and verify the meter reads -6.0 mV.
17. Calibrate the voltage offset for ±100 mV range: Move jumper JP3 to position B. Press the Offset switch to + (red) and adjust Voff 100 so that the meter reads +60 mV. Return the Offset switch to the Off position.
18. Balance the Clamp amplifier: Press the Function switch to clamp (red) and ensure that the Mode and Meter switches are set to Voltage (green). Adjust the Clamp trimpot so that the meter reads 0.0 mV.

This completes the calibration of a clamp channel. Repeat for each installed channel.

19. Calibrate Pulse Amplitude and DC Level: Final adjustment may be made for the Pulse amplitude and DC Clamp Level as follows. With the clamps set to voltage clamp as in step 18, press the Pulse On and press and hold Reset as above. Adjust the amplitude (R31) **{R20}** so that +10 mV is displayed on the digital panel meters. Press the amplitude gain to x10 and adjust R33 **{R21}** to read +100 mV for the clamped voltage. Turn the Pulse to Off and set the DC Clamp Level to + (Red) and the dial at 60.0 mV. Adjust R35 **{R22}** so that the clamped voltage reads +60.0 mV on the panel meter.

Specifications

Table IV: Specifications

Input Characteristics	
Input Impedance	$1 \times 10^{10}\ \Omega$
Common Mode Voltage	±13 V max
Common Mode Rejection	>120 dB
Drift	0.5 µV/°C max
Output Characteristics	
Compliance	±13V ±35V option HV
Speed	5 user selectable speeds

Offset	±10 mV, ±100 mV
Fluid Resistance	Ranges 0-100, 200, 500 or 1000 Ω or 01. and 10x these values depending on the current gain
Current Measurement Gain	1, 10 or 100 mV/µA
Panel Meter Voltage: Current: (gain setting dependent)	3 ½ digit LCD ±199.9 mV ±1999 µA ±199.9 µA ±19.99 µA
Power Requirements	100-130 VAC or 220-240 V, 50/60Hz fuse: 0.5A, 250V 0.315A, 250V Europe
Physical Dimensions VCC MC2 VCC MC6 VCC MC8 DM_MC6	W x D x H (cm) / Wt (kg) 23.3 x 26.7 x 14.6 / 4.0 43.7 x 26.7 x 23.5 / 8.3 59.1 x 26.7 x 23.5 / 10.2 9.0 x 3.7 x 4.1 / 0.16
Warranty	1-year parts and labor

Operating Conditions	Equipment is to be operated in a controlled laboratory environment. Temperature: 0-40 °C Altitude: sea level to 2000 m Relative humidity: 0-95%

DM_MC6 Input Module & Dummy Membrane

Description

The DM_MC6 is a combination electrode input interface and dummy test membrane for connection of single Ussing chamber setups to the VCC MCx multi-channel Voltage-Current clamps. This input device is better suited for use in systems where the chambers are physically separate from one another such as the EasyMount chamber systems. Electrodes are connected to the DM_MC6 via four pin tip sockets ((I1, V1, V2 and I2).

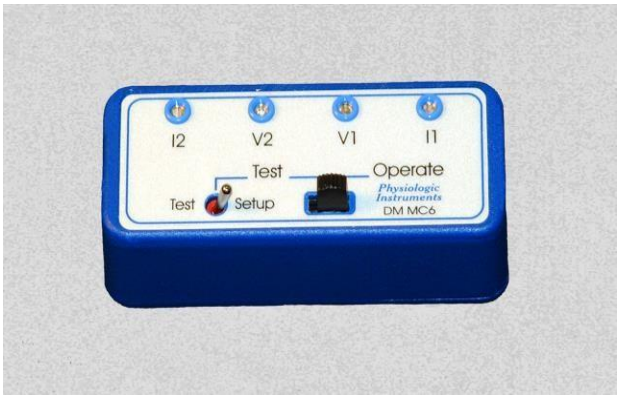


Figure 7: DM_MC6 Single Channel Input Module with integral dummy membrane.

Operation

A 4’ (optional 7’) shielded flat cable with modular plugs is provided to connect each DM_MC6 to the back of the VCC MCx clamp. The Operate - Test slide switch determines whether the clamp is to receive input from the chamber via the electrodes (Operate) or from the dummy membrane (Test). To make measurements from the Ussing chamber, push the slide switch to Operate to connect the electrodes to the input of the clamp and disable the dummy membrane circuitry.

The dummy membrane may be used at any time before or during an experiment to trouble-shoot problems and determine whether a problem resides in the electronics or the experimental setup. To use the dummy membrane, push the slide switch to Test and the toggle switch to Setup. "Test" isolates the electrode inputs from the clamp circuit and enables connection of a battery (AAA, 1.5 V) to the test membrane. "Setup” shorts the membrane resistance and is equivalent to setting up an Ussing chamber without a tissue. Fluid resistance compensation should be adjusted under this condition. Switching the toggle switch to "Test" connects the battery to the dummy membrane and is equivalent to mounting a tissue in the Ussing chamber. An open circuit voltage of approximately 25 mV and short-circuit current of ~80 □A should be observed. When finished with the dummy membrane push the slide switch to Operate to prevent run down of the battery.