

User's Manual

ECM[®] 2001 Electrofusion Systems



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- MA1 45-0080** ECM[®] 2001 Electro Cell Manipulator
(100 to 120 VAC)
- MA1 45-0080INT** ECM[®] 2001 Electro Cell Manipulator
(200 to 230 VAC)

BTX[®]

HARVARD APPARATUS

The Electroporation Experts

WEEE/RoHS Compliance Statement

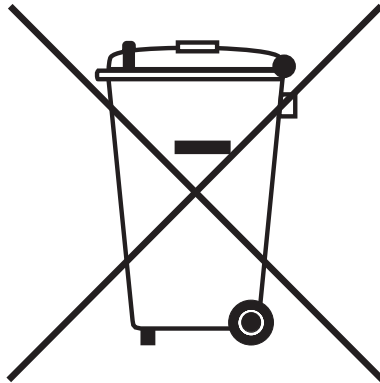
EU Directives WEEE and RoHS

To Our Valued Customers:

We are committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for our products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive - Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of our products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. We will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.



- **Do Not Dispose Product with Municipal Waste.**
- **Special Collection/Disposal Required.**

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General Information

Serial Number

The serial number for the ECM® 2001 is located on the rear of the instrument case. All inquiries concerning these products should refer to the serial numbers on the units.

Calibration

There is no calibration required for the ECM® 2001.

Warranty

BTX - Harvard Apparatus warrants the ECM® 2001 for a period of two years from the date of purchase. At its option, BTX - Harvard Apparatus will repair or replace the unit if it is found to be defective as to workmanship or materials. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than BTX - HARVARD APPARATUS without BTX - HARVARD APPARATUS' express and prior approval, (c) used in violation of instructions furnished by BTX - HARVARD APPARATUS. This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL BTX - HARVARD APPARATUS BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. **THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.** Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you.

Without limiting the generality of the foregoing, BTX - HARVARD APPARATUS shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence.

Warranty is void if the ECM® 2001 is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX - HARVARD APPARATUS.

Warranty is void if parts, connections or cell fusion chambers not manufactured by BTX - HARVARD APPARATUS are used with the ECM® 2001.

General Information (continued)

If a defect arises within the warranty period, promptly contact BTX – Harvard Apparatus, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number **1-800-272-2775** (US Only) or **508-893-8999** (E-mail: techsupport.btx@harvardapparatus.com). Goods will not be accepted for return unless an RMA (Returned Materials Authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device.

This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

Service

All service under the warranty will be made at the BTX - HARVARD APPARATUS, Holliston, Massachusetts facilities or an authorized service site. Owner will ship instrument prepaid to Holliston, Massachusetts, USA or the service site. BTX - HARVARD APPARATUS will return the instrument after servicing, freight prepaid to owner's address.

Obtaining Service:

Service During Warranty

1. Write or call the BTX - HARVARD APPARATUS Customer Support Group and describe the nature of the problem.
2. Carry out minor adjustments or tests as suggested by BTX - HARVARD APPARATUS.
3. If proper performance is not obtained, BTX - HARVARD APPARATUS will notify you to ship the instrument, prepaid, to its Service Department. The instrument will be repaired and returned at no charge for all customers in the continental United States.

Customers outside of the continental United States who have purchased our equipment from distributors should contact the distributor. If you have purchased your equipment from us, you should contact us directly. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost.

Note: Under no condition should the instrument or accessories be returned without prior approval from BTX - HARVARD APPARATUS. An RMA (Returned Materials Authorization) number must be obtained.

General Information (continued)

Out-Of-Warranty Service

Proceed exactly as for Warranty Service, above. If our Service Department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on the basis of labor and materials. A complete statement of time spent and materials used will be supplied. Shipment to BTX - HARVARD APPARATUS should be prepaid. Your bill will include return shipment freight charges.

Disassembly by the user is prohibited. Service should only be carried out by experienced BTX - HARVARD APPARATUS technicians.

Repair Facilities and Parts

BTX - Harvard Apparatus stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We offer complete reconditioning service.

General Safety Summary

Please read the following safety precautions to ensure proper use of your ECM® 2001. To avoid potential hazards and product damage, use these products only as instructed in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

To Prevent Hazard or Injury

OBSERVE ALL TERMINAL RATINGS

Review the operating manual to learn the ratings on all connections.

GROUND THE ECM® 2001

This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making any connections to the input or output terminals of the product, ensure that the product is properly grounded.

MAKE PROPER CONNECTIONS

Make sure all connections are made properly and securely.

DO NOT OPERATE WITHOUT COVERS

To avoid electric shock or fire hazard, do not operate these products with the covers removed.

AVOID EXPOSED CIRCUITRY

Do not touch exposed connections when power is present.

USE PROPER LINE CORD

Use only the specified line cord for this product and make sure line cord is certified for country of use.

USE PROPER FUSE

Use only specified fuses with product.

DO NOT OPERATE WITH SUSPECTED FAILURES

If damage is suspected on or to the products, do not operate. Contact qualified service personnel to perform inspection.

DO NOT OPERATE IN EXPLOSIVE ATMOSPHERE

To avoid injury or fire hazard, do not operate these products in an explosive environment.

DO NOT OPERATE IN WET/DAMP CONDITIONS

To avoid electric shock, do not operate these products in wet or damp conditions.

OBSERVE ALL WARNING LABELS ON PRODUCT

Read all labels on the products to ensure proper usage.

DO NOT CONTINUALLY PULSE UNIT

If you continue to pulse the unit without a 10-20 second delay between pulse series, the unit may overheat and result in damage to components.

<p>CAUTION FOR RESEARCH USE ONLY NOT FOR CLINICAL USE ON PATIENTS</p>

General Safety Summary (Continued)

DO NOT OPERATE IN LOW IMPEDANCE

Sample: Load or Sample

If the electroporation samples have an impedance of less than 20 Ω , the samples may arc and result in sample loss and potential damage to unit.

Safety Terms and Symbols:

Terms that appear in this manual:



WARNING. Warning statements identify conditions or practices that could result in injury or loss of life.



CAUTION. Caution statements identify conditions or practices that could result in damage to these products or other property.

Symbols that may appear on the products:



Danger
High
Voltage



Attention
Refer to
Manual



Protective
(Earth)
Terminal



Functional
Ground

Electrical & Technical Specifications

Standard Capabilities:

Operating Temperature	10° to 40°C
Storage Temperature	10° to 50°C
Humidity	90% relative humidity
Altitude	2,000 m (operating)
Pollution Degree	2 (Not to be operated in conductive pollutants atmosphere)
Power Requirements	100 to 120 VAC, 50/60 Hz 200 to 230 VAC, 50/60 Hz
Input Power	85 W maximum (idle) 1.6 kW maximum (peak)
Fusing	T, 8 A, 250 V 5 x 20 mm

Mechanical Characteristics:

Weight	22 kg (47 lbs)
Dimensions, L x W x H	43.2 x 39.4 x 28 cm (17 x 15.5 x 11 in)

Electrical Characteristics:

Alignment:

Frequency	1 MHz, fixed
Voltage	0 to 75 VRMS
Duration	0 to 99 sec

Post-Fusion AC:

Frequency	1 MHz, fixed
Voltage (VRMS)	1/10 of the alignment AC amplitude, attenuates with time
Duration	0 to 9 sec

Electroporation:

High Voltage Mode (HV Mode):

Voltage	10 to 3000 V peak
Pulse Length	1 to 99 µsec

Low Voltage Mode (LV Mode):

Voltage	10 to 500 V peak
Pulse Length	1 to 99 msec 0.01 to 0.99 msec

Number of Repeats	0 to 9
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Electrical & Technical Specifications

(continued)

Note:

LV Mode

Less than a 20% V drop at the end of the pulse with the following constraints:

PL ≤ 25 ms, load 3 20 Ω

PL ≤ 1 sec, load 3 1k Ω

PL ≤ 10 sec, load 3 10k Ω

HV Mode

Less than a 10% V drop at the end of the pulse with the following constraints:

PL ≤ 600 μs, load 3 40 Ω

BTX instruments are designed for in vitro and in vivo animal and plant applications only and are not for human Clinic.

General Specifications

Certifications and Compliances

Overvoltage Category:

CAT III Products in this Category: Distribution-level mains, fixed installation

CAT II: Local-level mains, applications and portable equipment

CAT I: Signal levels in special equipment or parts of equipment, telecommunications and electronics

Meets requirements of Directive 89/336/EEC for Electromagnetic Compatibility (EC) and Low-Voltage Directive 73/23/EEC for Product Safety.

Compliance was demonstrated to the following specifications as listed in the Official Journal of the European Communities:

EN 50081-1 Emissions

EN 55011 Class B Radiated and Conducted Emissions

EN 55082-1 Immunity

IEC 10004-2 Electrostatic Discharge Immunity

IEC 10004-3 RF Electromagnetic Field Immunity

IEC 10004-4 Electrical Fast Transient/Burst Immunity

Low Voltage Directive 73/23/EEC

EN 61010-1 Safety requirements for electrical equipment for measurement, control and laboratory use.

<p>CAUTION FOR RESEARCH USE ONLY NOT FOR CLINICAL USE ON PATIENTS</p>
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Introduction

The ECM® 2001 is a multifunctional electrofusion and electroporation square wave generator. Embryo and cell fusion are facilitated by the combination of the AC and DC wave pulses. Fusion is achieved by the generation of an AC current wave form that generates a benign dielectrophoretic alignment of cells. With only microsecond switchover time from AC to DC, efficient fusion takes place. After fusion, the AC is reapplied maintaining the cell compression for the rounding off process resulting in a higher number of hybrids.

ECM® 2001 features include:

- AC waveform of 1 MHz (optimal for fusion)
- Electroporation capabilities
- A wide range of voltages from 5 to 3000 V
- Finer voltage discrimination
- Capable of operating at low impedance loads of 20 Ω

Operation: Getting Started

Unpacking

The ECM® 2001 Electro Cell Manipulator System is shipped in a carton specifically designed to provide maximum protection for the instrument during transportation under normal handling conditions. Upon receipt, open the carton and carefully remove the ECM® 2001. The contents should be examined for any apparent damage resulting from shipment, and if necessary, BTX - HARVARD APPARATUS should be informed in writing. Be sure to save the carton and packing materials for any future transportation and shipping requirements.

Packing Data

Check the packing slips to ensure that all items ordered and listed are included in the shipment. If any parts are missing, call BTX - HARVARD APPARATUS at 1-800-272-2775 (US Only) or 508-893-8999, (E-mail: techsupport.btx@harvardapparatus.com) or your distributor.



CAUTION

Power Source

As received the ECM® 2001 is ready for use with either a 100 to 120 VAC, or 200 to 230 VAC, single phase, 50/60 Hz power source according to the electrical requirements of the country to which it is shipped.

The power cord has a standard three-prong plug. For safety reasons, it is mandatory that the instrument be grounded.

DO NOT MODIFY THE PLUG.

Installation

Once you have determined that none of the ECM® 2001 components are damaged or missing, proceed with the installation. The location of the ECM® 2001 should be a dry, level surface, free from extremes in ambient temperature, dust, or chemical exposures. Provide a minimum of a 6" space between the back and any obstruction to airflow while a 2" space on both sides of the unit is required for adequate airflow.

Unpack cables, chambers and accessories ordered and refer to "OPERATION" for further instructions.

Operation: Getting Started (continued)

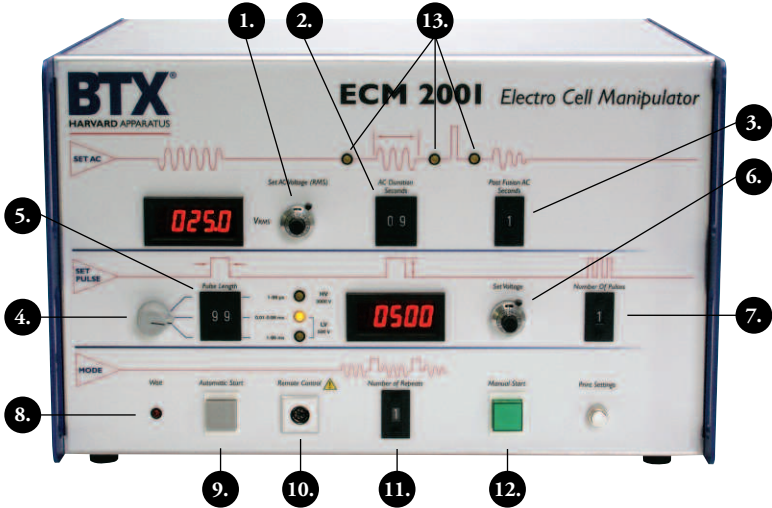
Location Requirements:

- A sturdy, level, clean, non-flammable, dry surface
- Adequate power source
- Ambient room temperature of 15° to 40°C
- Relative humidity of 20 to 80%, non-condensing
- A non-explosive atmosphere
- Adequate clearance for ventilation

Lifting & Carrying

Handles are provided at each side of the front panel for lifting and carrying the unit.

Quick Start



Front Panel Controls (See Instrument above)

# / Name	Function
1. SET AC VOLTAGE	This knob adjusts the AC alignment voltage from 0 to 75 volts RMS. The voltage is shown on the display to the left of the knob.
2. AC DURATION	The thumbpot controls the length of time that the SECONDS alignment is activated during the Automatic Mode. The time is set using the + and - push buttons to select any time up to 99 seconds.
3. POST FUSION AC	The thumbpot adjusts the AC duration after the SECONDS fusion pulse in the Automatic Mode. Simply set the pulse length up to 9 seconds for the compression of cells after the fusion.
4. SELECT MODE	This knob selects the electroporation mode between the HV mode (10 to 3000 V / 1 to 99 μ sec), LV mode (10 to 500 / 0.01 to 0.99 msec) OR LV mode (50 to 500 / 1 to 99 msec). Once the selection is made, the appropriate light will come on.

Quick Start (Continued)

# / Name	Function
5. PULSE LENGTH	The electroporation/electrofusion pulse length thumbpot controls the pulse length of the squarewave pulse. The pulse is set using the lower (+) and upper (-) push buttons to the “ready” mode.
6. SET VOLTAGE	This knob adjusts the pulse voltage. If the generator is in the HV mode, the voltage display (left monitor) is in Volts and it can be set from 10 to 3000 V in the HV Mode and 10 to 500 in the LV Modes. Turn clockwise to increase, counter clockwise to decrease the voltage.
7. NUMBER OF PULSES	Set number of pulses from 1 to 9. The + and - push-buttons are used to select the desired number of pulses. The yellow light illuminates when each pulse is delivered to the chamber and a beep is emitted.
8. WAIT	This light illuminates when the instrument has reached the end of an operating cycle and discharges all the capacitors for safety. The generator cannot be operated when this light is on. CAUTION: Do not change settings or initiate another cycle until wait light has extinguished.
9. AUTOMATIC START	Press and release to initiate an alignment and electroporation cycle according to the preset voltage, pulse length, and number of pulses.
10. REMOTE CONTROL	Optional Accessory – Connect the remote control box cable so the generator can be operated from the remote control box.
11. NUMBER OF REPEATS	The thumbpot can be used to set the number of repeat cycles. It can be set up to 9 cycles.

Quick Start (Continued)

<u># / Name</u>	<u>Function</u>
12. MANUAL START	This two-position button allows manual control of the ECM® 2001. Push IN to Start the alignment. The button illuminates and the alignment voltage is applied continuously until the button is pushed a second time to release or a timeout of 99 sec is reached. When released, the green light goes out and the electrofusion pulses are delivered to the chamber. If the timeout is reached, the AC waveform process LED changes from continuously on to flashing.
13. PROCESS LEDS	These LEDs illuminate to indicate each stage of the process.

Quick Start (Continued)

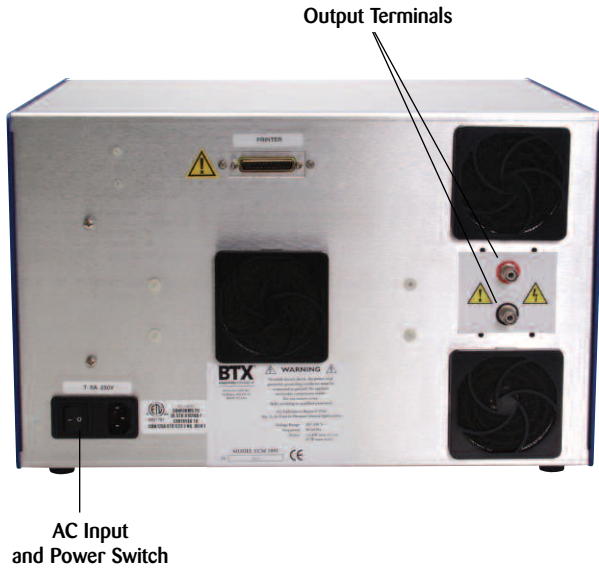
External Connections:

Output Terminals:

Type	SHV Coaxial
Voltage Rating	5000V DC Max

Remote Control:

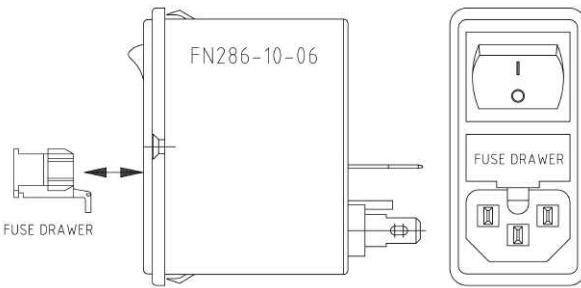
Type	Mini-Din, 6-pos, Female	
Pinout	Pin	Signal
	1	N/C
	2	RTN
	3	+12V
	4	Auto Start
	5	Manual Start
	6	N/C



Quick Start (Continued)

Replacing Power Entry Module Fuse

1. Turn power switch to the "OFF" position
2. Remove the Power Cable from the wall outlet
3. Remove the Power Cable from the ECM® 2001
4. Remove Fuse Holder Drawer (Refer to illustration)
5. Remove both fuses and perform a visual inspection or a continuity test on each fuse
6. Replace defective fuses with 8 A, 250 V, Time Lag (Slo-Blo) 5 x 20 mm Fuses (Refer to Spare Parts, pg. 32 for ordering information)
7. Install Fuse Holder Drawer (refer to illustration)
8. Install the Power Cable into the ECM® 2001
9. Plug Power Cable into wall outlet
10. Turn power switch to the "ON" position



Additional Fusing (Not Operator Replaceable)

Fuse Rating	Location
T 0.1 A 250 V	XFMR T1 Secondary 2
T 0.1 A 250 V	XFMR T1 Secondary 4
T 1.6 A 250 V	XFMR T1 Secondary 1
T 2.0 A 250 V	XFMR T1 Secondary 3
T 3.15 A 250 V	XFMR T2 Primary 1
T 3.15 A 250 V	XFMR T2 Primary 2
T 4 A 250 V	XFMR T4 Secondary

Operating Basics

Automatic Start Mode Operation

1. Connect Chamber to the input located in the back panel of the ECM® 2001.
2. Plug the ECM® 2001 power cable into correct line voltage outlet.
3. Switch POWER ON in the Back Panel.
4. Set AC Voltage (1) to desired voltage.
5. Set AC Duration Seconds (2) to desired time period.
6. Select between the HV or LV mode (4).
7. Set DC Pulse Length (5).
8. Set DC Voltage (6).
9. Set Number of Pulses (7).
10. Set Post-Fusion AC Seconds (3) to the desired time period.
11. Load BTX Chamber with cell suspension/reagents.
12. Check all settings.
13. Press Automatic Start (9), a beep is emitted and the ECM® 2001 delivers all preset parameters to the Chamber.
14. For electroporation only - set AC Voltage (1), AC Duration (2) and Post Fusion AC (3) to ZERO. Follow all other steps.

Manual Start Mode Operation

1. Follow Steps 1 to 13 in Automatic Operation
2. Press Manual Start Button (12) (manual start button illuminates and the AC waveform is output). In this mode, the AC Duration is over-ridden and the AC field is on continuously until a 99 sec time out occurs or the button is released. While viewing cells in a microslide under an inverted microscope, the AC Amplitude may be varied to optimize alignment.
3. When cells are aligned appropriately, press Manual Start Button again to release the AC and initiate the set DC parameters.

Operating Basics (Continued)

Selection of Operating Mode

Automatic Start of the fusion process is initiated by pressing the Automatic Start button (9); in this case, the pre-set AC Voltage and AC Duration is applied. Alignment is followed automatically by electroporation settings of DC PULSE Voltage and Pulse Length.

Manual Start (Best with Microslides viewed under a microscope). Push MANUAL START button (12) to turn on the alignment AC voltage. It will illuminate in this position. By pushing it in a second time, the AC alignment voltage is turned off and electroporation pulses are delivered to the test chamber.

Alignment Parameters

AC Voltage: Dial the voltage setting required using the AC Voltage(1).

AC Pulse Length: Dial the AC Pulse Length on thumbpot AC Duration switch (2).

Electroporation Parameters

Choose between HV or LV Mode (4).

Pulse Length: Dial the pulse Length on DC Pulse Length switch (5).

DC Voltage: Select the electroporation pulse Voltage by Set Voltage (6).

Number of DC Pulses: Set Number of pulses (7).

Post Fusion AC Parameters

Set the Post Fusion AC (3) time for compression of cells after electrofusion.

The post fusion AC amplitude is 1/10 of the alignment AC amplitude. The post fusion AC amplitude attenuates with time.

Operation With Microslides & Microscope

To connect Microslides to the ECM® 2001 pulse generator, attach Banana-to-Micrograbber Adapters to each HV cable and then connect the micrograbbers to the microslide contacts. It is advisable to tape the cables to the microscope stand to avoid the weight of the cables moving the Microslide. The Microslide wire electrode gap can be filled and emptied with a micropipette tip or a syringe with a hypodermic needle.

Operating Basics (Continued)

Operation With Microslides & Microscope (continued)

Adjust the AC voltage setting so that cells move slowly toward the Microslide Electrodes. Increase fusion voltage and pulse length in small increments (10%) to get increased fusion yield. Cells will drift apart after alignment if the fusion pulse is absent or too weak. Look for fusion events close to the wires. The electric field is strongest close to the wires and fusion will occur here first. If the field is too strong, the cells will burst (lyse) immediately.

If the cells do not move and an increase in voltage leads to boiling of the media, indications are that the cell solution acts too much like an electrolyte. Use fresh mannitol to prepare 0.3 M mannitol (0.2 micron filtered) with a pH of 7.0 to 7.3. Adjust pH with sterile 0.1 N NaOH. Measure pH of mannitol just prior to fusion. Other low molecular weight sugars such as sucrose and glucose have also been used successfully.

When operating Microslides under a microscope, it is advantageous to use the generator in the Manual Mode. This way, DC fusion pulses can be initiated as soon as alignment is achieved, thus subjecting the cells to minimal electrical stress. Use a stopwatch to measure the required alignment time. Set the same time or longer in the automatic mode when working with larger chambers. If a demonstration for several people is intended, a microscope with a TV camera attachment and monitor display serves well.

Advanced Operation: Programming

Important Notices

Please refer to the following important notices prior to setting up and using the ECM® 2001.



CAUTION

Manual Switch

Prior to turning on the ECM® 2001, verify that the GREEN manual push button switch is in the "out" or "Off" position, and that it is not pushed in. If the instrument power is turned on and the GREEN manual switch light comes on, turn it off immediately. Only use the manual mode with appropriate buffers under the appropriate conditions for AC alignment optimization.



WARNING

Procedure

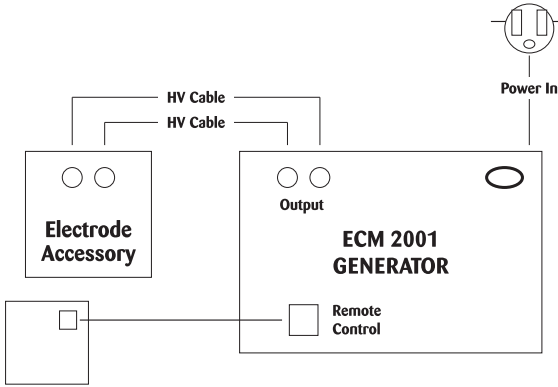
WARNING HIGH VOLTAGE: For practical and safety reasons do not touch any of the cables or electrode connections while the ECM® 2001 is in the Manual Start or Automatic Start Modes and the AC field or DC pulses is in use. Check that the unit is not operating and is in standby mode when connecting and disconnecting cables.

Advanced Operation: Programming (continued)

Equipment Setup

Setup of the ECM® 2001 Independent of Monitoring

Please refer to the following diagram for a graphical representation of the set up procedure for using the ECM® 2001:



Remote Control Box Accessory

(Optional)

1. Connect the ECM® 2001 into the appropriate line voltage outlet with the power cable.
2. Connect electrode accessories to the HV output jacks on the rear of the unit using recommended HV cables and adapters (See Appendix A).
3. Proceed to Operation.

Experimental Methods

Experimental Methods for Various Fusion Applications

The following suggested procedure is a recommendation only and each investigator is encouraged to experiment with alternate cell densities, fusion media and pre and post-fusion procedures, and consult relevant publications. Ohnishi et al (BTX ECM ID# 218) and Ruzin (BTX ECM ID#,183) describe successful protocols for mammalian hybridoma production

List of Materials

Cells:

1. Myeloma in log phase growth.
2. Spleen, peripheral blood cells, etc.

Fusion Solutions

Sterile 0.3 M mannitol, glucose or related sugars; 0.1 mM Ca^{2+} and 0.1 mM Mg^{2+} , pH 7.0 to 7.3 (adjust pH with 0.1 N NaOH.)

Fusion Facilitators (elective)

Trypsin at a concentration of 5 mg/ml sterile without salt or Pronase at a concentration of 1 mg/ml sterile without salt, or Calmodulin 100 g/ml and Glyceril mono-oleate 50 µg/ml.

Equipment & Other Materials

1. RPMI 1640 or DMEM with 10% fetal bovine serum and 2 mM L-glutamine.
2. Trypan blue to determine viability of cells before and after fusion.
3. Sterile 96 microwell plate.
4. Sterile pipette.
5. Microscope with 10x and 40x objective.
6. Optional ice baths for reagents.
7. Laminar flow hood.
8. Table top centrifuge.
9. Sterile conical tubes.
10. Availability of Autoclave.

Suggested Pretreatment of Mouse Cells for ECF

Spleen cells should be removed from the mouse and teased apart in cold Hank's Balance Salt Solution (HBSS) or RPMI 1640. Transfer spleen cells to a sterile test tube and allow the large particles to settle.

Pipette suspended spleen cells into a new sterile test tube. Remove erythrocytes. Preparations and cell concentrations for human cells and other fusion partners are the same.

Experimental Methods (continued)

Fusion Protocol

Establishment of optimal fusion parameters may take some time and must be done for each new myeloma/lymphocyte pair. Usually fusion is done at room temperature, but, as an alternative method one might elect to keep cell suspension on ice during processing. Additionally, one might decide to place fusion chambers in an ice bath during the actual fusion process. Immediately prior to fusion, the cells must be concentrated and washed in a nonelectrolyte solution.

Fusion In Standard 0.3 M Mannitol

The two cell lines are combined at a 1 to 4 myeloma/lymphocyte ratio and the mixture is centrifuged at 1,000 rpm for 6 minutes, to obtain a cell pellet. The supernatant is withdrawn and sterile 0.3 M mannitol with a pH between 7 to 7.3 is added.

The cells are washed two times in 0.3 M mannitol and resuspended to a final concentration of 2×10^6 cells per ml for fusion. Cells in 0.3 M mannitol should not be allowed to remain in solution for more than 2 hours.

Therefore, during the course of the experiment it may be necessary to prepare several cell pellets.

Fusion In Glucose

As an alternative to standard 0.3 M mannitol, one may elect to use other non-electrolytic buffers such as 0.3 M glucose at a pH of 7 to 7.3 for fusion. In this case one simply substitutes the sugar (or other substance) for the mannitol with the exception that the cells may be kept in the glucose for up to 4 hours.

Fusion Facilitators (elective)

One might add pronase or trypsin to remove some of the membrane surface proteins and to neutralize possible surface electric charges. This is advisable if cells are difficult to fuse.

Post-Fusion Protocol for Standard 0.3 M Mannitol, Glucose & Related Sugars

1. Following the fusion process, remove cell suspension under sterile conditions from the fusion chamber and carefully pipette into a sterile test tube. Add 10 ml of RPMI 1640 or DMEM with 10% fetal bovine serum and 2 mM L-glutamine. Allow to rest for 15 minutes.
2. Centrifuge cells at 500 rpm for 5 minutes and remove supernatant. Carefully resuspend cells in culture medium and pipette cells into a 96 microwell plate at a concentration of 2×10^5 cells per well (0.2 ml per well). Check wells for sufficient amount of medium.
3. Incubate cells at 37°C with 5% CO₂ overnight.

Experimental Methods (continued)

4. The following day, remove one-half of the medium and re-feed cells with HAT medium. On days 4, 7, and 10, again remove one-half of the medium and re-feed with HAT. Cells may be supplemented with feeder layers or other growth factors.
5. Check cultures daily for HAT medium selection. Once colony formation is observed, begin feeding with HAT medium at first phase of expansion.

Plant Protoplast Fusion

The following protocol is intended only as a guide to researchers involved in the genetic engineering of plant protoplasts.

Basic Procedure

1. Obtain freshly isolated protoplasts as per usual methods. (**Note:** Fusion will not be successful after cell wall regeneration has occurred.)
2. Gently pellet in non-electrolytic solution.
3. Remove supernatant and resuspend in non-electrolytic solution, adjusting the density to 1.5 to 6 x 10⁵ protoplasts/ml according to size.
4. Proceed with electrofusion.

Solutions

In general, optimal viability will occur when non-electrolytic solutions are most consistent with pre-fusion solutions in terms of osmotic potential, pH, and composition.

1. Non-electrolytic sugars such as mannitol, glucose, sorbitol, etc. are recommended.
2. Low-salt concentrations of CaCl₂ (140 M) and Hepes (600 M) are optional, and, over a limited time, do not appear to be detrimental.
3. pH settings can be explored for each protoplast type. Fusions have been most successful at pH values of 7.0 to 7.5.
4. Osmotic potential should be equal to the protoplast isolation solution.
5. Recalcitrant protoplasts have been successfully fused by the addition of two drops (per ml) of trypsin (Sigma Typ XIII PCK treated, 5 mg/ml). Other facilitators such as pronase, dispase, DMSO, PEG, etc. should also be investigated.

Experimental Methods (continued)

Instrument Settings

Typical fusion yields should average approximately 20% with the addition of protease, and 7% without. PEG treated cells fuse typically at a 1% yield.

1. Addition of more than 1 or 2 pulses appears to damage protoplasts without increasing yield.
2. Typical settings for the Microslide might be as follows:

	Amplitude	Time/Pulse Width
AC ALIGNMENT	035 V	25 sec
DC FUSION	260 V	40 µsec

Yield: Total Fusions 8.6%

Heterokaryons 2.6%

Nuclear Transfer/Cloning

The following protocol is intended only as a guide to researchers involved in nuclear transfer/cloning applications. This protocol was adapted from Meng et al, 1977 (BTX ID#3588)

ElectroSquarePorator™ T820 ELECTROFUSION PROTOCOL CELLS: rhesus monkey oocyte

Cell Preparation

1. Prepare enucleated recipient oocyte and donor blastomere as indicated in the paper.
2. Prepare monolayer culture with buffalo liver cells (BRL 1442), CMRL-1066, and 10% neonatal calf serum. Maintain monolayer culture at 60% confluency during coculture, and change medium every 24 hours.

Electroporation Settings

Choose Mode	HV (99msec/3 kV)
Set Voltage	130 to 140 V
Set Pulse Length	50 msec
Set Number of Pulses	2 pulses
Chamber	BTX microslide P/N 450 (0.5 mm gap)
Desired Field Strength	2.6 to 2.8 kV/cm

Experimental Methods (continued)

Electroporation Procedure

1. Aspirate an individual blastomere into a microinjection pipette and place under the zona pelucida of an enucleated oocyte or zygote.
2. Culture in KSOM with 10% NCS.
3. Expose oocyte pairs to KSOM containing cycloheximide (7.5 mg/ml), cytochalasin B (7.5 mg/ml), and 10% NCS for 1 hour to induce ooplast activation. Then place them individually in an electric field.
4. Induce membrane fusion by 2 continuous square wave DC electrical pulses in 0.3 mannitol, 100 mM CaCl₂, 100 mM MgSO₄, and 0.5 mg/ml polyvinylpyrrolidone (with the aid of a pipette controlled by a micromanipulator or just manually by hand).
5. Oocyte pairs receive 2nd and 3rd pulses at hourly intervals. Between pulses, hold the pairs in KSOM, cytochalasin B, and cycloheximide.
6. Reconstituted embryos are subsequently cocultured on a BRL cell monolayer.

Results

3 biochemical pregnancies resulted with one lost at 30 days of gestation. The other two culminated in the births of one male and one female monkey. Genetic typing indicated that the putative sire (sperm donor) and the putative mother (female from which the nucleus was harvested) were the true biological parents of both nuclear transfer infants.

Reference: Meng et al, Rhesus Monkeys Produced by Nuclear Transfer, *Biology of Reproduction*, 57, 454-459, 1997 (DB # 3588)

Step-By-Step Procedures To Optimize Yield of Fusion Products

After preparing the cell lines for fusion as described above, the following procedures will aid in the determination of optimum instrument settings. This is important if new cell lines are to be fused for which no previous experimental data is available.

- Determine instrument setting with Microslide
- Set both AC Voltage (1) and DC Voltage (6) to zero.
- Set AC (2) and DC (5) time periods to zero.
- Fill wire gap with cell suspension.
- Increase the AC voltage to 10 V.
- Push Manual Start button (12) and observe cells for 30 seconds. If no movement occurs, increase AC Voltage by increments of 10 V until movement is observed.

Experimental Methods (continued)

- Change cell suspension between experiments, if heating or drying is observed.
- Turn off the alignment signal if cell suspension is changed, by pushing Manual Start (12) button (99 second limit) a second time. Signal is on as long as the AC waveform process LED is on continuously (not flashing).
- Experiment with AC Voltage and time period until a good percentage of the cells gently form dimers (doublets) within 10 to 20 seconds. Note settings.
- Set AC time period to the value found good for alignment.
- Set the Post Fusion AC (3) time period up to 9 seconds to compress the cells after electrofusion.
- Optimize system parameters with respect to cell viability. Use the basic settings that were derived earlier and make several runs with modified settings. Cultivate cells for 4 days from each run in RPMI 1640 or DMEM, 10% fetal bovine serum, hypoxanthine and thymidine 2 mM L-glutamine and test part of the cell culture each day for viability by trypan blue exclusion. Choose settings which lead to highest viability after 4 days.
- System parameters which should be investigated for cell viability are as follows:
 - Vary Alignment Voltage and duration in steps of 20% Higher voltage requires shorter duration.
 - Vary DC Voltage and Pulse Length in steps of 20%.
 - Experiment with Post Fusion AC duration to hold cells together after fusion.

Troubleshooting

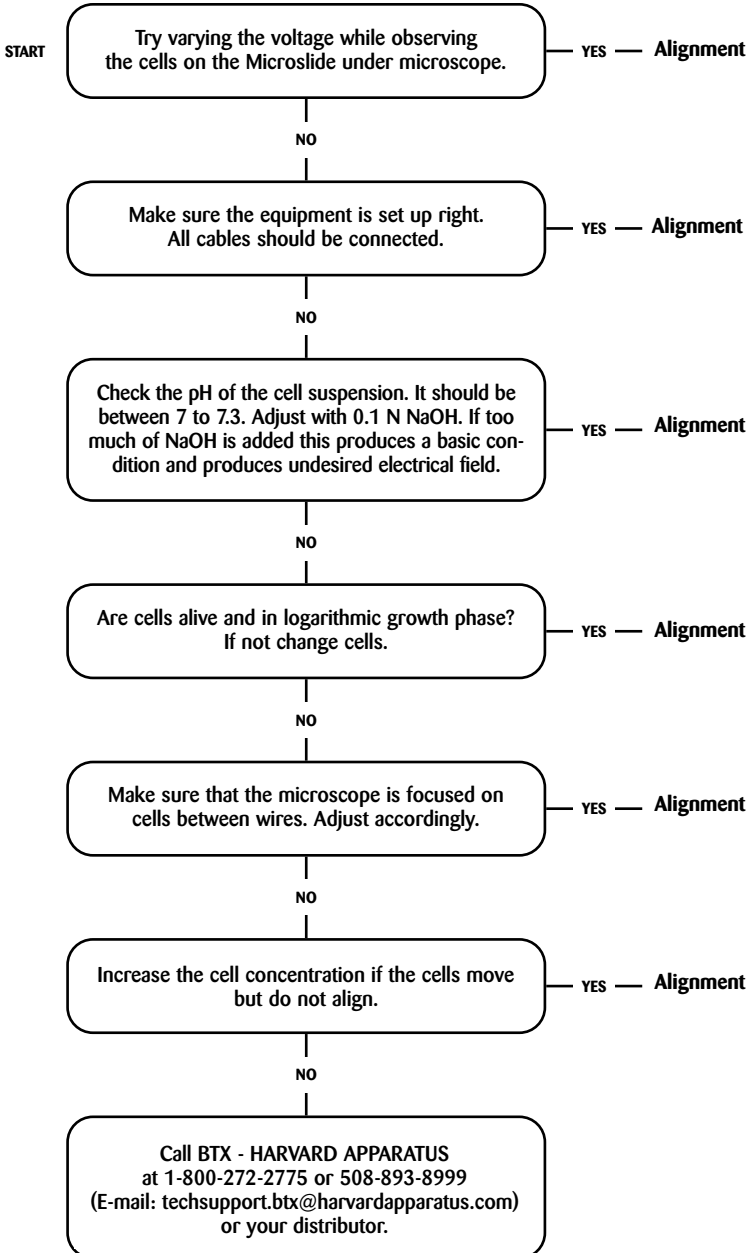
For trouble shooting refer to Appendix A:

- Cells Don't Align
- Cells Don't Fuse
- Cells Don't Divide
- Cells Don't Secrete Antibody

Appendix A: Troubleshooting

Cells Do Not Align

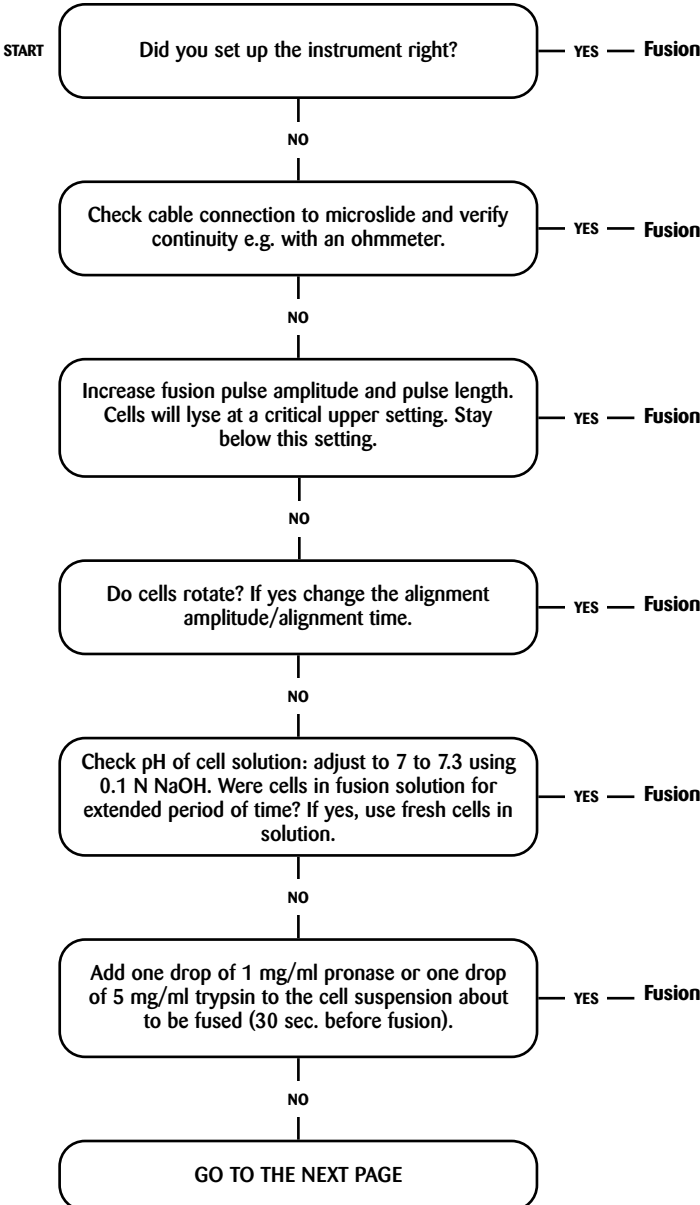
Make sure the Microslide is clean (free of cell debris)



Appendix A: Troubleshooting (Continued)

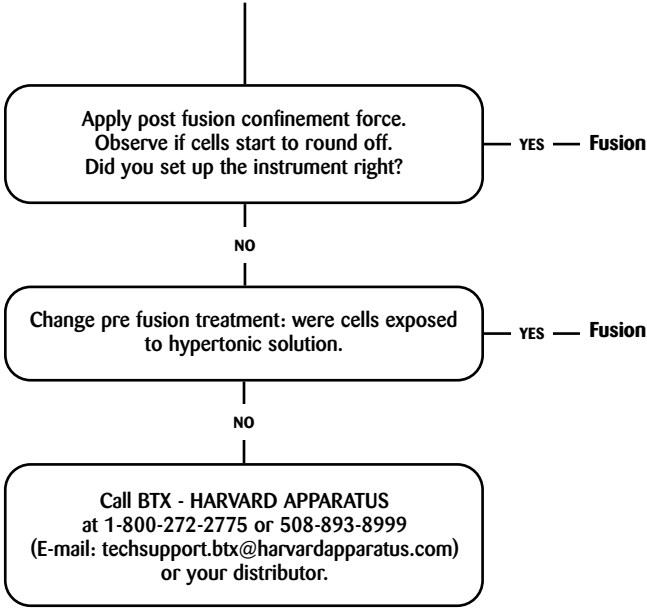
Cells Do Not Fuse

Observe the microslide under microscope. Fusion is successful if cell pairs do not drift apart after the fusion pulse and when they round off slowly.



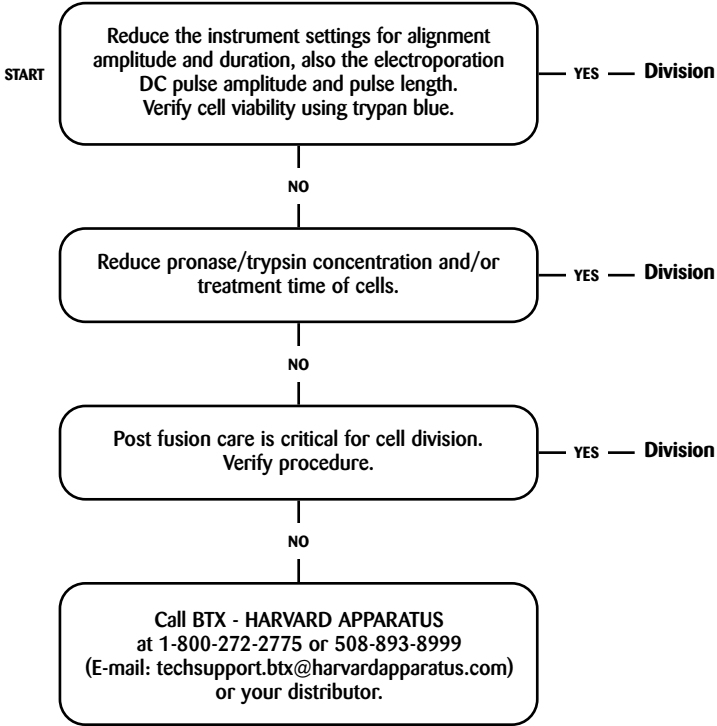
Appendix A: Troubleshooting (Continued)

Cells Do Not Fuse (continued)



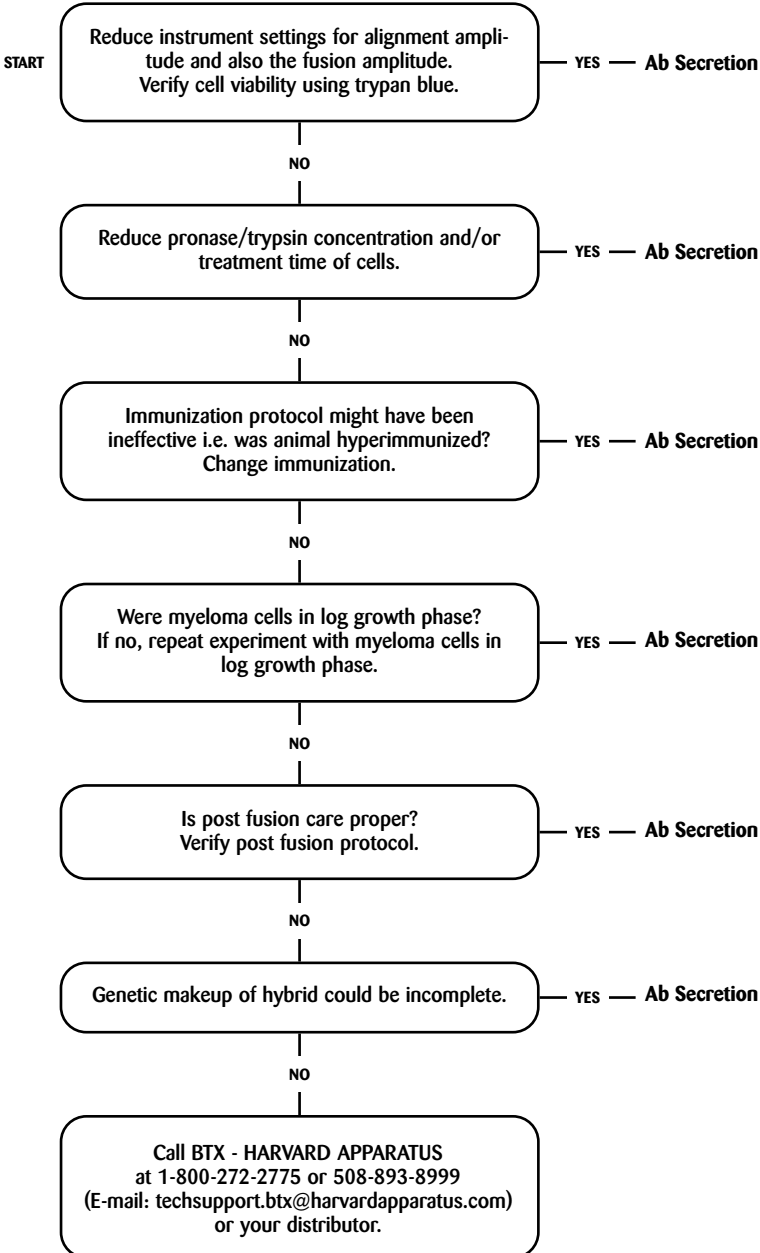
Appendix A: Troubleshooting (Continued)

Cells Do Not Divide



Appendix A: Troubleshooting (Continued)

Cells Do Not Secrete Antibody (Ab)



Appendix A: Troubleshooting (Continued)

Generator Test

#	Generator Test	Yes	No
1.	Make sure the power cord is connected to the ECM® 2001, and it is plugged in.		
2.	Turn on the power. Do the displays illuminate?		
3.	Set the AC Voltage Setting (1) and DC Voltage Setting (6) to Zero. Do the monitors display Zero?		
4.	Set Select Mode Knob (4) to the HV mode 1 to 99 μ sec. Does the HV Mode green light illuminate?		
5.	Set Select Mode Knob (4) to the LV Mode 0.01 to 0.99 msec. Does the LV Mode green light illuminate?		
6.	Set Select Mode Knob (4) to the LV Mode 1 to 99 msec. Does the LV Mode green light illuminate?		
7.	Push in the Manual Start button. Does the manual start button light illuminate?		
8.	Push in the Manual Start button again. Does the manual start button light extinguish?		
9.	Set the following parameters on the instrument <ul style="list-style-type: none"> • Set the AC Voltage (1) setting to 30 V. • Set the AC Durations (2) to 3 seconds. • Set the Post Fusion (3) to 3 seconds. • Set the Select Mode Knob (4) to HV Mode 1 to 99 μsec. • Set the Pulse Length (5) to 40. • Set the DC Set Voltage (6) to 50 volts. • Set the Number of Pulses (7) to 2. • Press the Automatic Start (9) button and release. Do the process LEDs (14) illuminate?		

Please contact BTX - HARVARD APPARATUS Technical Services at

(800) 272-2775 or **(508) 893-8999**

E-mail: techsupport.btx@harvardapparatus.com

if you answered "no" to any of the questions above.

Appendix B: Glossary of Technical Terms

Alignment

A consequence of cells being exposed to an inhomogeneous or divergent electric field, resulting in their movement toward the electrodes, and subsequent alignment or pearl chain formation.

Alternating Current (AC)

During electro cell fusion, an alternating electrical current is utilized to induce an inhomogeneous, or divergent electric field, allowing for dielectrophoresis and pearl chain formation. The resulting physical contact between cells facilitates the fusion process that may result following the application of a DC electroporation pulse.

Capacitor

A device that stores energy in the form of an electric field. A capacitor consists of two metal plates insulated from each other by a dielectric (insulating, usually a plastic material such as Mylar) material. In an ideal capacitor, no conduction current flows between the plates after the capacitor is completely charged. Capacitors can be fixed, variable, or adjustable.

Chambers

Electroporation and Electrofusion Chambers are the devices used to contain the cells/molecules to be fused/transfected. The chambers include the electrodes through which the ECM® 2001 pulse generator delivers the electric field to the cells. Fusion Chambers include the Microslides (Model 450, 450-1, 453, 453-10), Meander Fusion Chamber Model 454, and the Flat Electrode Model 484.

Cloning

In terms of applications for electro cell fusion, cloning refers to the ability to generate identical, viable animals, through processes such as nuclear transplantation.

Compression

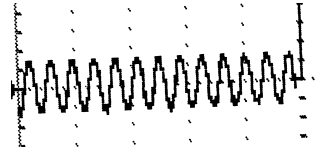
See Alignment, Dielectrophoresis, and Pearl Chains. The result of an AC alignment.

Dielectric Breakdown

The reversible breakdown of bi-lipid layer membranes as a result of the application of a DC electroporation pulse. A sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane.

Dielectric Constant

For a given dielectric (nonmetallic) material, the ratio of electrical capacitance of a dielectric-filled capacitor to a vacuum capacitor of identical dimensions.



Alternating Current (AC)



Disposable Electroporation Cuvettes Plus™

Appendix B: Glossary of Technical Terms (continued)

Dimer Formation

The bringing together of two cells, through the process of dielectrophoresis, so that they may be fused, resulting in a hybrid. Refer also to Pearl Chain.

Direct Current (DC)

During electro cell fusion, a direct current is utilized to produce a high intensity, short duration electric field, causing a reversible physical breakdown of bi-lipid membranes and resulting in the formation of temporary pores. When juxtaposed pores in the membranes of two or more cells reseal following the DC pulse, cells may become fused. See Electric Field.

Frequency

The number of times an oscillation goes through a complete cycle in one second. The unit is either cycle/sec or Hertz (Hz).

Homogenous Electric Field

An electric field where the direction and strength of the field lines are constant.

Divergence

The deviation of field lines (e.g. electric field lines) from parallel, homogeneous conditions. A highly divergent field is a very inhomogeneous field where the value and direction of the field change drastically in the area under consideration.

Electric Field/Electric Field Strength

The potential difference between two points (electrodes) (in Volts) divided by the distance between the electrodes (called gap, and expressed in cm). Expressed as V/cm or kV/cm. This is true only if the electric field is homogenous as it is in parallel plate electrodes.

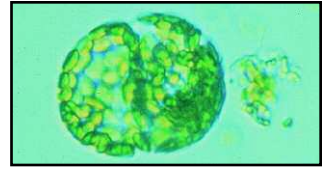
Electric Field Force

The force acting on any charge in an electric field. The force is equal to the product of the charge and the electric field.

Electrofusion (EF) or Electro Cell Fusion (ECF) Electric field induced cell fusion. A novel physical means to bond two cells together by the application of an electric field pulse of high intensity.

Electrolytic

A fluid containing charged molecules is called an electrolyte. Electrolytic properties are associated with such a fluid, such as the ability to conduct current.



Dimer Formation



Direct Current (DC)

Appendix B: Glossary of Technical Terms (continued)

Electroporation

The application of high electric field pulses of short duration to create temporary pores (holes) in the membranes of cells.

Embryo Manipulation

The cloning of animals can be accomplished through embryo manipulation techniques, such as nuclear transfer and electrofusion.

Frequency

The number of times an oscillation goes through a complete cycle in one second. Unit is Hertz (1/sec).

Homogenous Electric Field

The direction and field strength are constant.

Hybrid

A viable daughter cell resulting from the fusion of two parent cells.

Hybridoma

The fusion of an antibody producing cell with an immortalized cell, resulting in an immortalized hybrid cell capable of generating monoclonal antibodies.

Hydrostatic Pressure

The pressure in liquids at rest.

Inhomogeneous Electric Field

Direction and strength of the electric field vary.

Microslide

An electrofusion chamber allowing for the observation of the alignment and fusion processes under a microscope.

Osmotic Pressure

The applied pressure required to prevent the flow of solvents of different concentration across a semipermeable membrane.

Pearl Chains

See Alignment and Dielectrophoresis. Chains of cells or vesicles brought into alignment during electro cell fusion, prior to electroporation.

Pore

A small, mostly transient opening in a cell membrane/wall caused by the application of a brief high electric field pulse.

Appendix B: Glossary of Technical Terms **(continued)**

Potential Difference

The difference (in Volts) between points in an area between electrodes.

Pressure Gradient

The difference in pressure between two points in a medium.

Pulse Length

The length of time an electric signal is applied.

Relaxation Time

The time of a system to reach equilibrium.

Rounding Off

The phenomena of cells forming a sphere after being fused together.

Turgor Pressure

The pressure in capillaries.

Voltage

The difference of electric potential between two electrodes (expressed in volts (V) or kilovolts (kV)).

Wave Forms

The shape of time varying electric signals.

Appendix C: Accessories and Replacement Parts

Accessories

Catalog Number	Model	Description
MA1 45-0082		Cable Set, HV, 1 meter
MA1 45-0083		Cable Set, HV, 3 meter
MA1 45-0085		Cable, Remote Control, 2 meter
MA1 45-0086	ECM2001R	Remote Control Box
MA1 45-0087		Adapter Set, Banana-to-Minigrabber
MA1 45-0088		Adapter Set, Banana Splice, F/F
MA1 45-0089		Adapter Set, Banana-to-Square Post Cables
MA1 45-0090		Adapter Set, Banana-to-Pin Tip, F/F
MA1 45-0207	630B	Safety Stand
MA1 45-0058	Enhancer 3000®	Enhancer 3000® Electroporation Monitoring System
MA1 45-0100	366	Petri Dish Electrode, 2 mm Gap
MA1 45-0101	384	Caliper Electrode, 1 x 1 cm, Brass
MA1 45-0102	384L	Caliper Electrode, 1.5 x 1.5 cm & 2 x 2 cm, Stainless Steel
MA1 45-0103	450	Microslide, 0.5 mm Gap
MA1 45-0104	450-1	Microslide, 1.0 mm Gap
MA1 45-0105	453	Microslide, 3.2 mm Gap
MA1 45-0106	453-10	Microslide, 10 mm Gap
MA1 45-0107	454	Meander Fusion Chamber
MA1 45-0108	484	Flat Electrode, 1mm Gap
MA1 45-0109	485	Flatpack Chamber, 1.83 mm Gap
MA1 45-0110	486	Flatpack Chamber, 0.56 mm Gap
MA1 45-0113	508	Genetrode, Straight, 5 mm
MA1 45-0114	510	Genetrode, Straight, 10 mm
MA1 45-0115	512	Genetrode, Bent, L-Shaped, 5 mm
MA1 45-0116	514	Genetrode, Bent, L-Shaped, 3 mm
MA1 45-0117	516	Genetrode, Bent, L-Shaped, 1 mm
MA1 45-0118	520	Tweezerrode Electrode, 7 mm diameter
MA1 45-0119	522	Tweezerrode Electrode, 10 mm diameter
MA1 45-0122	542	Genepaddles, 3 x 5 mm
MA1 45-0123	543	Genepaddles, 5 x 7 mm
MA1 45-0124	610	Cuvette, 1 mm Gap, pkg. 50
MA1 45-0125	620	Cuvette, 2 mm Gap, pkg. 50

Appendix C: Accessories and Replacement Parts (continued)

Catalog Number	Model	Description
MA1 45-0122	542	Genepaddles, 3 x 5 mm
MA1 45-0123	543	Genepaddles, 5 x 7 mm
MA1 45-0124	610	Cuvette, 1 mm Gap, pkg. 50
MA1 45-0125	620	Cuvette, 2 mm Gap, pkg. 50
MA1 45-0126	640	Cuvette, 4 mm Gap, pkg. 50
MA1 45-0130	PP35-2P	Petri Pulser, 2 mm Gap
MA1 45-0203	515	Genetrode/Genepaddle Holder

Spare Parts

Catalog Number	Model	Description
06-004009-01		Fuse, T, 8 A, 250 V, 5 x 20 mm

Appendix D: General Cleaning and Maintenance

To clean exterior surfaces, use a soft, lint-free cloth to remove loose dust. For more efficient cleaning, use a soft cloth dampened with water or an aqueous solution of 75% isopropyl alcohol. Do not immerse the ECM® 2001. Avoid using abrasive cleaners. Avoid using chemicals containing benzene or similar solvents. To clean fan and vent filters, remove outer cover and then remove filter mesh. Rinse filter mesh with water to remove dust and allow to dry. Reinstall filter mesh and outer cover.

The ECM® 2001 requires no maintenance.

If the ECM® 2001 does not work properly, contact
BTX – Harvard Apparatus for appropriate instructions.

ECM® 2001 Generator Troubleshooting Guidelines

Basic Information

Date:

Customer Name:

Institution:

Serial Number:

Software Version (will be displayed as v. x.xx when you first power on the instrument):

Diagnostic Test

Please complete the following chart. Simply set the voltage, pulse length, pulse interval and pulse numbers on the ECM® 2001 as indicated, and record the actual feedback after pulsing. Use 400 µl of 1X PBS in a 4 mm cuvette as a 20 Ω load. Use 200 µl of 1X PBS in a 4 mm cuvette as a 40 Ω load. Make sure all cables are well connected and cuvettes make good contact with the safety stand to ensure proper current flow.

ECM 2001 Manual Troubleshooting Section

Please refer to Appendix A (page 29-34) in the ECM® 2001 manual for troubleshooting guidelines. Please contact BTX Technical Service if the ECM® 2001 routinely Arcs, or if you smell anything coming from the machine.

Mode	Set V (V)	Set PL	Set Pulse No.	Expected V (V)	Actual V (V)	Expected PL	Actual PL	Load (µL 1x PBS)
LV	500	1 ms	1	450-550		0.9-1.5 ms		400
LV	500	100 µs	1	450-550		90-110 µs		600
LV	500	10 µs	1	450-550		7-13 µs		400
HV	1000	100 µs	1	850-1150		90-110 µs		400
HV	3000	100 µs	1	2700-3300		90-110 µs		600

ECM® 2001 Generator Troubleshooting Guidelines (continued)

Additional Information

Please help us diagnose the problem by providing as much information as possible.

1. What is your main concern? Please describe in detail.
(Either instrument related or sample related. Please indicate past history for repairs and service if applicable.)
2. Please briefly outline your protocol below:
Cell line(s):
Set voltage:
Set pulse length:
Set pulse number:
Set pulse interval:
Mode: LV or HV (circle one)
Buffer (composition and volume):
Cell Density (cell/ml):
Transfectant Name (ie. DNA, RNA, protein, etc.):
Transfectant Amount:
Other information:
3. Are you following an existing protocol or publication? If so, please specify:

Note: If using BTX protocols, please indicate the protocol ID number on the bottom left corner.

Final Instructions

We appreciate your patience. Please DO NOT send the instrument back to BTX without prior authorization from the company. Instruments will not be received without a proper RMA (Return Material Authorization) number assigned.

When all the information is completed, please contact BTX Technical Service via one of the following methods:

- Email: techsupport.btx@harvardapparatus.com
- Fax: 508-429-5732
- Phone: 1-800-272-2775

Thank you and we will do our best to assist you!

BTX Technical Service Group