AgilePulse™ IM

In Vivo
Gene Delivery System

USER'S MANUAL

A Message from BTX

Thank you for investing in an AgilePulse system.

Since its founding in 1983, the main focus of BTX has been in the area of applying controlled electric fields for genetic engineering applications. Because of this, we quickly established a reputation as the technological leader in the fields of electroporation and electrofusion. Our systems have been installed in many prestigious institutes around the globe where they are used successfully for high efficiency transfection, transformation and cell fusion applications. We offer a variety of waveforms, electrodes and chamber options to provide you with the best tools to achieve your goals.

We are vested in your success. To that end, the BTX technical support team constantly tracks published literature for any reference to electroporation and electrofusion. We extract the pertinent experimental conditions and yields from these papers to help us in our efforts to help you. In addition to tracking publications, we are available to you for support at any time for advice in experimental design, product recommendations, troubleshooting, and any other relevant technical advice.

We thank you again for your investment and we look forward to assisting you in any way we can.

Finally, please read this manual carefully before attempting to operate the electroporation system. If you have any questions about the unit or about particular applications, please contact us:

> **BTX**
> 84 October Hill Road
> Holliston, MA 01746 USA
>
> Toll Free: 1-800-272-2775
> International Callers: 508-893-8999
> Fax: 508-429-5732
> Web: www.btxonline.com
> Email: support@hbiosci.com

For any customers outside the US or Canada, please call your local BTX dealer or call us directly.

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Warranty Information

RESEARCH ONLY

BTX
84 October Hill Rd
Holliston, MA 01746, USA

Phone: 1-508-893-8999
Fax: 1-800-429-5732
Web: www.btxonline.com

Warranty

BTX warranties AgilePulse systems for a period of two years from the date of purchase. At its option, BTX will repair or replace the product if it is found to be defective as to workmanship or materials. This warranty does not extend to any device which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than BTX without BTX express and prior approval, (c) used in violation of instructions furnished by BTX. This warranty extends only to the original customer purchaser. **IN NO EVENT SHALL BTX 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 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 BTX AgilePulse system is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX. Warranty is void if parts, connections or electrodes not manufactured by BTX are used with the BTX AgilePulse instrument. If a defect arises within the warranty period, promptly contact BTX, 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: support@ hbiosci.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.

Out of Warranty Service

Proceed exactly as for Warranty Service above. If our service department can assist you by phone or other correspondence, we will be glad to help 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 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 technicians.

Repair Facilities and Parts

BTX stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample photo or drawing.

Caution Notice

The BTX AgilePulse systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) and safety intended for laboratory equipment applications.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

Safety Information

Please read the following safety precautions to ensure proper use of your generator. If the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

To Prevent Hazard or Injury

Emergency Stop

If a problem occurs during a run, push the STOP/RESET button on the front panel.

Use Proper Line Cord

Use only the specified line cord for this product and make sure line cord is certified for country of use. The operating voltage range for the AgilePulse Series is 100 to 240 VAC, 50/60 Hz.

Ground the Product

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. Any signal wire connections to the unit must be no longer than three meters.

Observe All Terminal Ratings

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

Use Proper Fuse

Use only specified fuses with product.

Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

Do Not Operate with Suspected Failures

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

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to operate the disconnection device.

Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.

High Voltage Risk

These instruments contain a high voltage power supply adjustable to 1,000 V. High voltage power supplies present a serious risk of personal injury if not used in accordance with design and/or use specifications, if used in applications on products for which they are not intended or designed, or if they are used by untrained or unqualified personnel.

- The user must read this manual carefully before the instruments are placed into operation.
- Removing the cover will void the warranty.
- Do not connect or disconnect the high voltage cable with the high voltage enabled.
- To connect or disconnect the cable, turn line power off and unplug line (mains) cord.
- Do not touch the electrode tip while the waveforms are being applied.
- Do not under any circumstances turn the power switch off while the system is pulsing; if an emergency occurs push the emergency button on the front panel

If there are any questions about the operation of this instrument, call BTX Customer service at 1-800-272-2775, or 1-508-893-8999.

Caution Notice

The BTX AgilePulse Systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications as well as the applicable safety requirements for electrical equipment for measurement, control, and laboratory use. The unit itself does not generate waste, but may be used to treat samples that are hazardous. Please use appropriate PPE and ensure disposal in accordance with local regulations and practices.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

Caution	Protective Ground Terminal	Caution Risk of Electric Shock

AgilePulse™ IM System Components & Set-Up

Introduction

The AgilePulse™ in vivo system provides the capability of delivering DNA , RNA, proteins, drugs, or other transfectants of interest to skin or muscle tissue. The system is designed for easy set-up and operation.

Specifications are presented on page 25.

The AgilePulse IM system is composed of three subsystems:

- Touchscreen controlled AgilePulse Waveform Generator
- Intramuscular Array (IM) parallel, needle electrodes
- User Manual

Optional equipment available includes:

- Foot Pedal (47-0420)
- Adapter box for electrodes with 4mm banana cables (47-0090 or 47-0091, please see Ordering Information page 27 for details)
- 2-Needle Array, 5 mm gap, pkg. of 6 (45-0121)
- 2-Needle Array Handle, for 5 mm gap (45-0206)

Figure 1: AgilePulse Waveform Generator

The AgilePulse IM Waveform Generator

The Waveform Generator (Figure 1) is composed of a computer and high voltage pulsing circuits. The computer runs mobile Windows® and is operated by a touch screen. On the front panel there is a power switch, a touch screen, an emergency stop switch, an electrode connector and two USB ports. All User inputs are entered via the touch screen. The system may be operated by using an optional foot pedal. On the back panel there is a line/mains power connector.

The high voltage pulsing circuits are controlled by a microcontroller. The microcontroller accepts user inputs from the touch screen and produces the pulse waveforms. A second microcontroller is used as an independent audit of the waveforms produced. If the audit function detects a deviation from the desired pulse protocol, the system immediately terminates pulsing. The system also digitizes the output of the pulse voltage and pulse current monitors and calculates the skin resistance of the tissue on each pulse. This is a significant quality control function that indicates the vaccine is being delivered properly. After the vaccine is delivered, a log of all parameters is saved in Internal Memory and on a USB memory key, if one is inserted.

AgilePulse Waveform Generators come in two varieties, specialized for pulse accuracy in relation to electrical resistance of the sample tissue. AgilePulse ID is recommended for electroporation of high resistance tissue, such as skin and tibialis muscle. AgilePulse IM is recommended for low resistance tissue such as quadriceps muscle. Please refer to Figure 2, AgilePulse Waveform Generator Selection Guide, for details.

AgilePulse Waveform Generator Selection Guide		
Tissue Type	**Approximate Tissue Load Range**	**Recommended Waveform Generator**
Skin, intradermal electroporation	1100 to 2200 Ohms	AgilePulse ID
Tibialis muscle	800 to 1500 Ohms	AgilePulse ID
Quadriceps, small animal	200 to 400 Ohms	AgilePulse IM
Quadriceps, large animal	80 to 250 Ohms	AgilePulse IM

Figure 2

AgilePulse™ IM System Components & Set-Up

Front Panel

The following can be found on the front panel: the Mains/Line power switch (illuminated when on), the Emergency Stop button switch, two USB ports, the IM Electrode Connector, and the User Touch Screen (Figure 3).

One USB port can be used for a Memory Key. The data logs that result from a experiment are automatically stored on the Key if one is inserted. The other USB port may be used for the optional Foot Pedal.

The Emergency Stop button switch is used to stop system operation. This switch immediately stops all pulsing and turns off the high voltage power supply. The Line/Mains power switch should never be used to turn off the system in an emergency situation if pulsing is in progress.

Back Panel

On the back panel there is an IEC Power Entry module and provisions for an Ethernet port.

Ethernet connectivity is not currently available.

Intramuscular Array Electrode (IMA)

The IMA consists of two parts: the handle and the tip. The handle is connected to the waveform generator. The tip contains needle electrodes used to deliver the transfectant (DNA/vaccine/drugs etc.) into the target cells. The needle arrays are disposable, but can be used for multiple experiments provided proper cleaning, and care of electrodes, are followed, and sterilized.

The IMA electrode is described in more detail on page 8.

Figure 3: AgilePulse Front Panel

AgilePulse™ IM System Components & Set-Up

Setting Up the System

The system will be set up in the following sequence:

1. Unpack the contents of the shipping box.

2. Check for obvious signs of exterior damage. If damage is noted, contact BTX Customer Service before proceeding.

3. Place the Waveform Generator on the top of a sturdy table.

4. Connect the electrode cable into the connector at the bottom right of the front panel.

5. Connect the mains/power cord into the back panel at the bottom right.

6. Connect the foot switch (if applicable) into one of the USB ports at the bottom left corner of the Waveform Generator (front panel).

PLEASE CONTACT BTX IF YOU ARE INTERESTED IN AN ON SITE TRAINING.

Initial System Test

This section will describe the process to verify mains/line cable has been installed properly and the computer boots up.

1. Connect the system power cord to the mains. (Plug in the device.)

2. Turn the rocker switch (Line/Main Power) on the front panel to the "on" position (I).

The rocker switch labeled "Power" should illuminate, and the Login Screen should appear within 10 seconds. If the Waveform Generator power switch fails to illuminate, then return it to the OFF position. Verify the power cord is properly plugged into the wall. Verify the wall socket is functional. It may be necessary to check the fuse. See Maintenance section (page 26) for instructions, proper replacement of the fuse, and the correct fuse type.

If the power is properly applied, the screen should appear as that shown in Figure 4. After entering the user login information and tapping "OK", the software continues its initialization procedure and the NEXT button will have a white fill.

If the internal system checks are successfully completed, the NEXT button will turn GREEN indicating the system is ready for use (Figure 5). If the NEXT button turns RED, then the internal checks failed (Figure 6). The system cannot be used if the button is red. Contact BTX Customer Service for assistance.

This completes the setup and testing of the AgilePulse™ System. If there is a problem, contact Technical Support for assistance.

Phone **508-893-8999**, or toll free **800-272-2775**
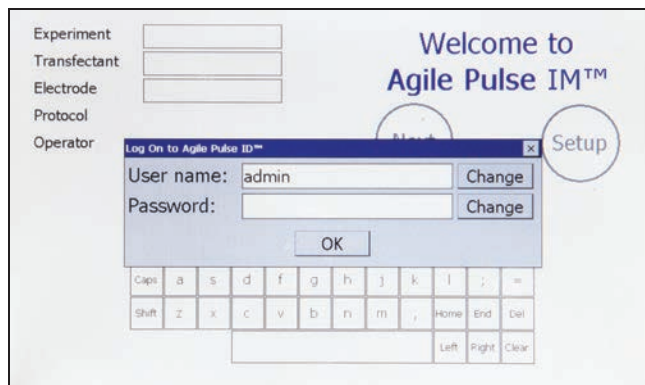
Website **www.btxonline.com**

Figure 4: Login Screen
(visible about 10 seconds after power on)

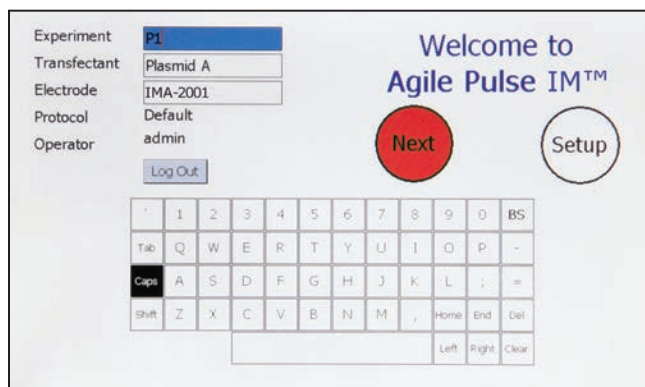**Figure 5: System OK Screen (Operating Correctly)**

Figure 6: Self Check Was Not Successful

System Operation

This chapter describes the procedure to operate the Waveform Generator and to use the Intramuscular Electrode.

Experiment Delivery

The user operates the system via the front panel touch screen. When the power is turned on, the system boots up, and displays the opening screen. The system is immediately ready for use. All data logs produced for a transfectant delivery will be automatically placed on a USB Key if one is inserted into an open slot on the front panel. The user must ensure the USB Key is inserted before the Protocol Delivery Screen is displayed, as the protocol pulsing is started.

If the user does not have a USB Key inserted at run time, all run history log files can be retrieved at a later time from the log files.

USB Key/Memory Stick

The USB key can be inserted into either of the two USB ports that are located on the front panel of the waveform generator. It should be inserted before delivering pulses and can be removed at any time after the "saving data" message disappears. The USB key may be removed without performing any additional procedures. The data logs that result from a experiment are automatically stored on the Key.

Figure 7: IMA Electrode Handle

Intramuscular Array (IMA) Electrode

The IMA Electrode consists of two parts: The Handle and the Tip.

IMA Electrode Handle

The handle consists of an assembly into which the tip is placed and a permanently attached cable. The cable is connected to the Waveform Generator. The handle is made of a durable plastic. It is not sterile and may be cleaned with alcohol. The handle is reusable.

Figure 8: IMA Tip

IMA Electrode Tip

The IMA tip is a disposable array. The tip is made of medical grade plastic with surgical steel needles. There are three tips that may be used with the handle.

The tips are shipped in individual pouches. A cover is included to protect the needles during shipping and handling.

When ready to use the electrodes, open the package and remove the tip.

Insert the tip into the handle by aligning the arrow on the tip with the arrow on the handle. Side grips have been added to the tip for easier application.

Once the transfectant is ready for administration, remove the protective cover from the IMA tip taking care not to disconnect the tip from the handle.

Figure 9: Attaching IMA Tip to Handle
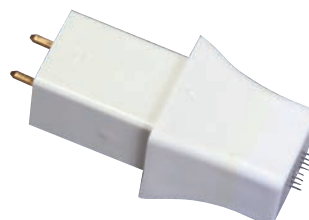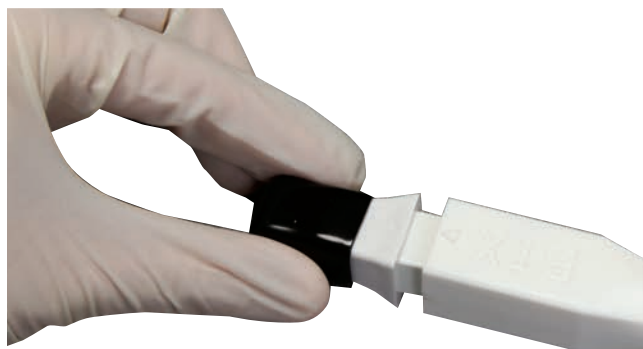
System Operation

Waveform Generator

Login Screen — User Identification Input

After power is turned on, a Login Screen is presented that requires the user to enter a user name and password combination. The Login system is designed to prevent unauthorized users from accessing the system and confine authorized users to permissible activities.

Two types of users are defined: system administrator and standard user. There is only one system administrator of the system who has the ability to add/remove pulse waveform protocols, add/remove standard user accounts, and assign pulse waveform protocol access privileges to standard users. The administrator always operates under the "admin" account.
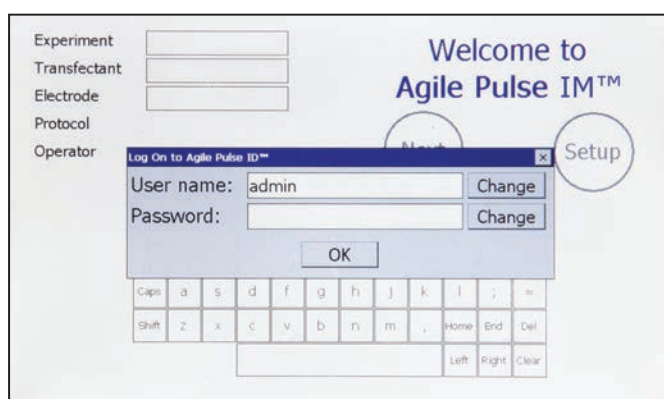

Figure 10: User Login Screen

Standard users are intended to be everyday users of the system, who only have the ability to run pre-defined pulse waveform protocols from an access list as determined by the administrator. The administrator may grant a standard user the ability to modify an already defined protocol, but not to add/remove protocols.

AgilePulse™ IM is factory configured with only the "admin" account available. By default no password is defined so the user can enter the system with parameters matching that of Figure 10 on first log in. The password may then be changed from the User account screen in Setup section. The most recently logged in user will be displayed in the User Name field on startup.

To enter User names and Passwords, touch the Change buttons to activate a virtual keyboard.

User name: Enter the user's name to log in

Password: Enter the password associated with the user account. Password entry is masked with "*" characters in order to provide increased security.

When the User name and Password have been entered, touch OK to log in.

Welcome Screen — Identification Input

An opening screen (Figure 11) allows input of key experimental information after the initial system log in. Information is entered by a virtual keypad. The purpose of this screen is for the USER (Researcher) to enter the Experiment IM, Transfectant and Electrode IM.

Figure 11: Opening Screen

All three parameters are set by user selected input. Touching a text field box highlights it blue and allows modification. Use the available touch screen keypad to enter up to 15 characters for each. Alternatively, a USB keyboard may be plugged into one of the front panel USB connectors and used to edit the ID fields manually. This screen cannot be operated from the foot pedal (ID fields cannot be selected or edited). The following pulse delivery screen may be operated by either the touch screen or by the optional foot pedal depending on the specification.

After verifying the identifications are correct go to the delivery screen by touching NEXT.

System Operation

Experiment Delivery Screen Function and Operation
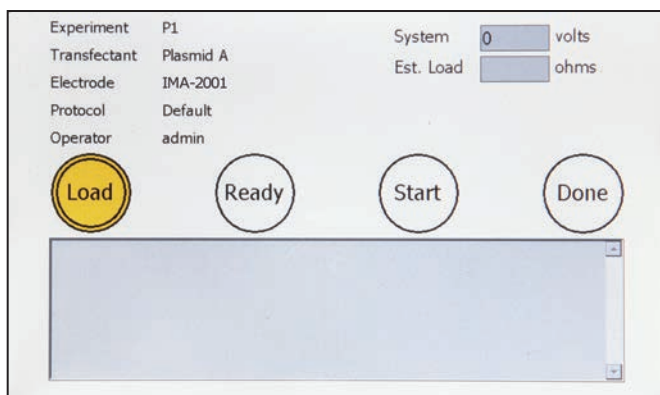
Figure 12: Delivery Screen — Initialization

The purpose of the delivery screen is to initiate pulses to deliver DNA to tissue. The procedure is as follows.

1. The researcher injects the transfectant into the muscle tissue of the animal, and inserts the electrode according to the approved protocol.

2. The researcher determines if the electrode is inserted properly by touching the LOAD button or by depressing the foot pedal. A number will appear in the Est. Load box. LOAD button will change to a green color. Estimated load in ohms is displayed. This number is the effective resistance of the animal's muscle and will continue to be updated every second.
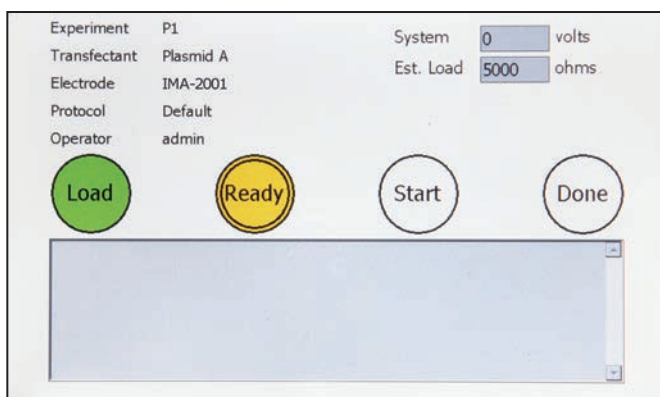
Figure 13: Delivery Screen — Load Estimation

3. If the LOAD Reading is within the range of 15 to 1500 ohms then the next step is to turn on the high voltage power supply by touching the READY button or depressing the foot pedal. The READY button will flash green until fully charged and then will stay solid green. The high voltage reading will appear in the System message box. If the researcher wants to abort the process, the electrode should be removed and the DONE button pressed. In this case, an early termination message will appear in the data log and the system reset.
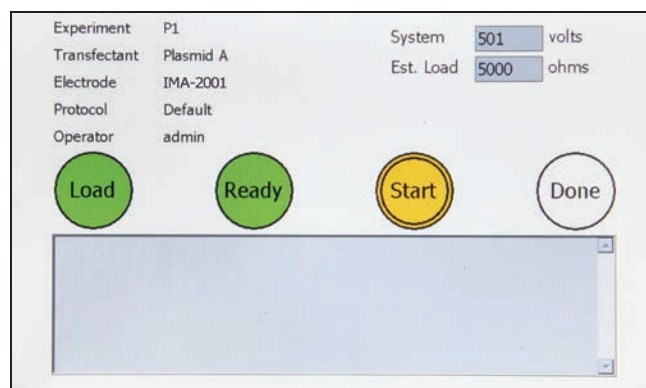
Figure 14: Delivery Screen — High Voltage Power Supply Charge

4. If the researcher determines that the system voltage is correct, pulsing is initialized by touching START or depressing the foot pedal. The START button turns a solid blue while pulsing is active and solid green when pulsing is completed.
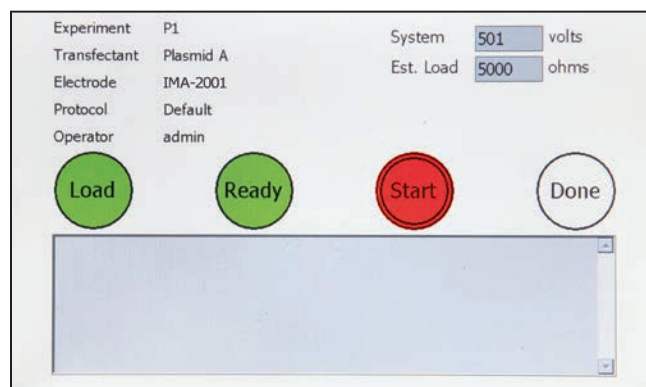
Figure 15: Delivery Screen — Pulse Waveform Active

5. When the system reaches a shutdown state either by successful waveform delivery or by error, all buttons will turn solid green and pulse delivery data will be displayed in the System message text box. The load message box will change from the estimated load value to the actual high voltage pulse monitored value.

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System Operation

6. All run history log data are automatically stored in internal Flash Memory as well as a USB Key, if one is inserted.
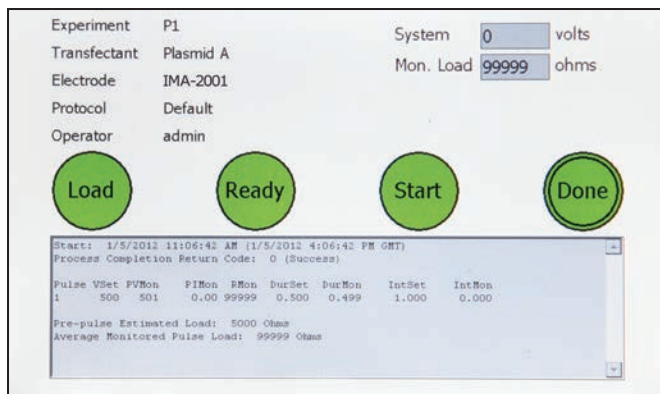
Figure 16: Delivery Screen — Shutdown

7. Press the color-filled DONE button to return to the opening screen.

Experiment Delivery Logs

All collected data from experiments are automatically stored in the internal Flash Memory and an inserted USB key under a unique chronological directory structure. Included items are an XML file containing raw detailed system and runtime data, a text log with basic runtime data, and a comma-separated CSV file containing pulse monitor data. The directory structure is constructed in a "AgilePulse\Log\<Year>\<Month>\<Day>\<Time>" hierarchy. A maximum of 20,000 delivery logs can be stored in the internal system memory.

	Raw Data (CCEP <Date> <Time>.xml)	Text Log (CCEP <Date> <Time>.log)	CSV Data (CCEP <Date> <Time>.csv)
Start and Stop Date	•	•	
Start and Stop Time	•	•	
Experiment	•	•	•
Transfectent	•	•	•
Electrode IM	•	•	•
Process Completion Type	•	•	
Set Pulse Parameters	•	•	•
Average monitor pulse voltage		•	
Average monitor pulse current		•	
Average monitor pulse resistance		•	
All monitor pulse voltage samples	•		•
All monitor pulse current samples	•		•
Monitor width of each pulse	•	•	•
Monitor interval of each pulse	•	•	•
Pre-pulse Load Reading	•	•	
System Serial Numbers	•		
System Software Versions	•		

System Operation

Text Log

The text log contains the same information that appears on the pulse delivery process screen after completion. Text log data is summarized in an easy-to-read format and is designed to provide quick feedback regarding the delivery completion. An example Log printout is given below in Figure 17.

Field	Description
Pulse	Pulse number
VSet	Voltage programmed to high voltage power supply
VMon	Voltage monitored during pulse
IMon	Current monitored during pulse
RMon	Equivalent load resistance calculated from voltage and current monitors R = V / I
DurSet	Pulse duration programmed to waveform generator
DurMon	Pulse duration monitored by independent time-auditor system
IntSet	Pulse interval programmed to waveform generator
IntMon	Pulse interval monitored by independent time-auditor system

```
Date Start:  8/14/2011    6:00:09 AM   (8/14/2011  1:00:09 PM GMT)
Experiment:    Pl
Transfectant:  Plasmid A
Electrode:     IMA-2001
Operator:      BTX

Final Completion Code Code:     0 (Success)
+++++++++++++++++++++++++++
Start:        8/14/2011    6:00:09 AM   (8/14/2011  1:00:09 PM GMT)
Process Completion Return Code:  0 (Success)


Pulse    VSet     PVMon    PIMon    RMon     DurSet    DurMon   IntSet    IntMon
1        450      450      0.351    1282     0.050     0.050    0.200     0.200
2        450      451      0.322    1401     0.050     0.050    50.000    49.856
3        110      110      0.141    783      10.000    9.975    20.000    19.944
4        110      109      0.146    750      10.000    9.975    20.000    19.944
5        110      108      0.147    736      10.000    9.975    20.000    19.944
6        110      108      0.141    767      10.000    9.975    20.000    19.944
7        110      108      0.132    821      10.000    9.975    20.000    19.944
8        110      108      0.131    827      10.000    9.975    20.000    19.944
9        110      108      0.159    681      10.000    9.975    20.000    19.947
10       110      109      0.144    756      10.000    9.975    20.000    0.000


Load Reading:      204
Date Stop:   2/12/2007  2:16:40 PM  (2/12/2007  10:16:40 PM GMT)
Time Elapsed:      00:00:18
```

Figure 17: Text Log Screen

System Operation

CSV Data

Detailed pulse information is exported from raw data and formatted to a standard comma separated file, primarily meant to be imported into Microsoft Excel® for further user manipulation. As an example in Figure 18, a user chart was made to display the monitored amplitude samples of three 100 microsecond pulses run at 5 kHz. In this case, each pulse contains eight amplitude samples.

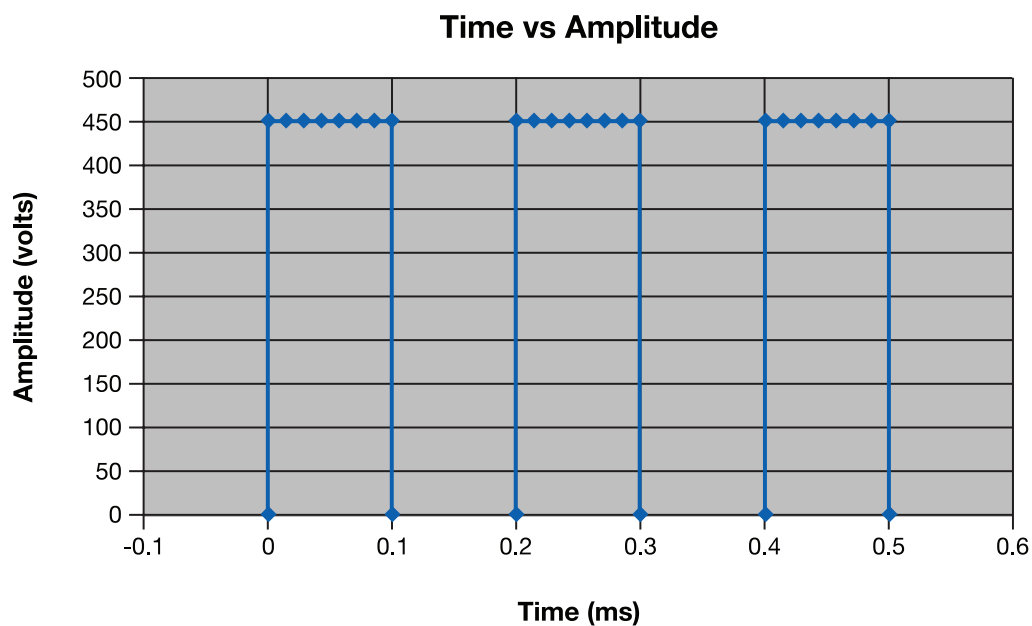**Time vs Amplitude**

[Chart: Amplitude (volts) on y-axis from 0 to 500, Time (ms) on x-axis from -0.1 to 0.6. Three pulses shown at approximately 450 volts, located between 0–0.1, 0.2–0.3, and 0.4–0.5 ms.]

Figure 18: Sample Amplitude Analysis

System Operation

Delivery Completion Return Codes

Return codes are available following system shutdown describing how it took place.
Please contact BTX technical support to resolve the errors.

Return Code	Description
0	Successful completion (Normal Operation)
1	Emergency Flash memory storage failure
2	Waveform protocol data integrity verification error
3	Waveform protocol mode not recognized
4	Protocol voltage below minimum specification
5	Protocol voltage above maximum specification
6	Protocol pulse width below minimum specification
7	Protocol pulse width above maximum specification
8	Protocol pulse interval below minimum specification
9	Protocol pulse interval above maximum specification
10	Protocol pulse number below minimum specification
11	Protocol pulse number above maximum specification
12	Protocol has a zero pulse group in between two non-zero pulse groups
13	Protocol pulse settings exceed maximum duty cycle
14	Low voltage power supply failure (Hard system turn-off)
15	High volt power supply voltage above maximum voltage
16	High volt power supply maximum current draw
17	Pulse voltage monitor detected off-zero voltage while no pulse was active
18	Pulse voltage monitor below maximum droop specification
19	Pulse voltage monitor above maximum overshoot specification
20	Pulse current monitor below minimum specification
21	Pulse current monitor above maximum specification
22	Independent time-audit system failed to respond to query
23	Independent time-audit system detected less pulses than expected
24	Independent time-audit system detected more pulses than expected
25	Independent time-audit system exceeded maximum measurable pulse number
26	Independent time-audit system detected excessive pulse duration variance
27	Independent time-audit system detected excessive pulse interval variance
28	Independent time-audit system data integrity verification failure
29	Reset button on front panel triggered
30	Control system watchdog timeout
251	High voltage timeout
252	Load estimation timeout
253	Delivery process was terminated prematurely
254	Control system experienced a system reset of unknown source
255	Load estimate outside of allowable range

System Operation

Software Maintenance Mode

The authorized system administrator is provided with the means to set system access privileges, add and delete pulse protocols, and access delivery log files. Non-administrator standard users can modify pulse protocols if given access by the administrator and retrieve previous experimental delivery logs. To enter this mode, press the Setup button on the startup screen.

User Account Maintenance

The user account maintenance screen is accessed from the Welcome screen:

Touch SETUP

Touch USER

User accounts and privileges are managed from this screen. While standard users will only see the Active User section, and thus will only be able to change their password, the system administrator has access to account properties for all standard users.

As an administrator, a new user account can be adding by pressing the "Add New User" button. Once the account is created the new user is assigned read-only access to all of the pulse protocol waveforms on the system. The administrator may then select individual protocols and provide more access by giving both read and write privileges, or they may restrict access to a protocol altogether by giving the user no access at all. When a user is not given any protocol access they will not be able to see or select it from the Protocol screen when they login under their own user name. When protocols are added through the Protocol screen after a standard user account has already been created, all user accounts in the system will receive read-only access to the new protocol.

The administrator also has the ability to change a user's password. This is ideal for situations in which the user forgets their password and thus need the password to be reset in order to login again.
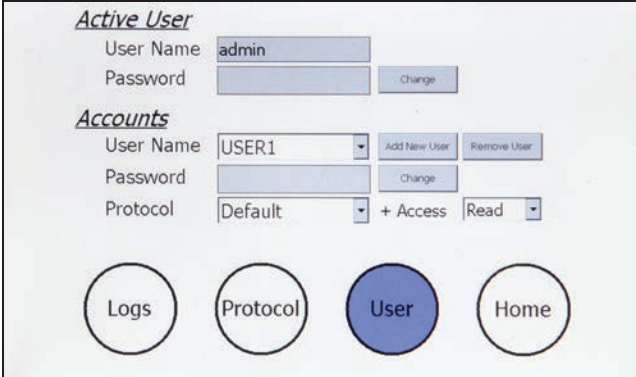

Figure 19: User Account Screen

IMPORTANT! The changes made on this screen will only be permanently saved when the HOME button is pressed to go back to the Welcome Screen. If modifications are made and system power is turned off while still viewing the screen, all changes will be lost.

Caution

System Operation

Log Retrieval

All previous experimental delivery logs can be retrieved and saved onto a USB Key or deleted from internal memory. The interface consists of a series of chronological sub-folders from which the user is allowed to double-tap on the touch screen to descend through the hierarchy. The standard naming sequence is Year, Month, Day, followed by special folders with the time of day. These time folder icons show a hand underneath, meaning they contain all files generated from a single transfectant delivery. The " " folders allow the user to return to the previous level of hierarchy.

Special time folders display basic information on the right hand side of the screen when selected. Identification numbers, time of day, and status completion indicators help the user find the correct vaccine delivery run to download.

When selected by a single tap on the touch screen icon, any level of hierarchy can be saved to a USB Key inserted in the front panel by pressing the SAVE button. The hierarchy structure will be maintained and copied to the USB Key. The DELETE button is also available to remove the hierarchy from internal memory. This delete is permanent and the lost files cannot be recovered. A maximum of 20,000 delivery logs can be stored on the internal system memory.

DEPENDING ON THE NUMBER OF FILES TO BE DELETED, THIS PROCESS CAN BE SLOW.

Figure 20: Log Retrieval Screen

Pulse Protocol

The pulse protocol to be delivered at run time is set by pressing the PROTOCOL button and adjusting parameters via the touch screen interface. Parameters for three pulse groups are modifiable by selecting a box and scrolling through the available values. Subsequent groups are activated by selecting more than one pulse in the number field and conversely deactivated by selecting zero pulses in the number field.

The adjusted protocol is saved to internal memory when the HOME button is pressed. Minimum and maximum parameter values are as follows:

Figure 21: Pulse Protocol Modification

Intragroup					
Pulse Amplitude	Pulse Width	Number of Pulses	Duty Cycle	Pulse Interval	Load
50 to 1000 volts	50 to 1000 μs	10	50%	1 to 1000 ms	15 to 1500 ohms
50 to 300 volts	0.050 to 10 ms	10	50%	1 to 1000 ms	200 to 1500 ohms

* For Pulse Amplitudes greater than 300 V, the maximum allowable Pulse Width is 1 ms

** Pulse duty cycle is limited to 50%.

⚠ **Caution**

IMPORTANT! The changes made on this screen will only be permanently saved when the HOME button is pressed to go back to the Welcome Screen. If modifications are made and system power is turned off while still viewing the screen, all changes will be lost.

AgilePulse™ IM Tutorial

Applications

The AgilePulse™ IM system is used to deliver molecules of interest to muscle tissues for research purposes only. The AgilePulse IM system has been shown to increase DNA delivery to cells resulting in 100 to 1000 times higher protein expression compared to hypodermic needle injection alone.

Experimental Delivery Methods

There are several methods used to deliver transfectants of interest.

System Name	Company	Mechanism	Target	Factors (all pain ratings are estimates)
AgilePulse ID AgilePulse IM	BTX	Inject transfectant in dermis or muscle with hypodermic needle, insert needle array, apply electric field to deliver into cells	Dermis/Muscle	• Large dose • Reduced pain compared to other electroporation systems • Pain 3 out of 10 • High cell transfection
PMED™	PowderMed	Microscopic particles coated with DNA, delivered into cells using compressed helium	Epidermis	• Small doses • Possible tattooing • Pain 3 out of 10 • Low/Medium cell transfection
Biojector® 2000	Bioject Medical Technologies	Inject using CO_2 pressure with spacer (subcutaneous) or w/o spacer (to muscle)	Skin or Muscle	• Large doses • Pain 3 out of 10 • Medium cell transfection
ECM 830, Gemini X2, and others	BTX and others	Inject into muscle insert electrode and apply electric field	Muscle	• Large dose • Significant pain • Pain 5 to 6 out of 10 • High cell transfection
Many	Many	Deliver viral vector with hypodermic needle into muscle	Muscle	• Immune system reacts to viral vector, inefficient when boosting • Pain 2 out of 10

AgilePulse™ IM Tutorial

Delivery Into Cells Using Electroporation

General Electroporation Discussion

Electroporation is the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. Their presence allows molecules, ions, and water to pass from one side of the membrane to the other. As the right side bar shows, when the electric field is applied the ions inside and outside the cell membrane migrate. As the charge builds up on either side of the membrane the membrane weakens and the pathways form permitting material outside of the cell to enter. If the electric field is promptly removed the pathways close and the membrane reseals. If the electric field duration is too long the pathways increase and the cell is killed. Efficient electroporation depends on proper selection of electric field waveforms. The electropores are located primarily on the membrane areas which are closest to the electrodes. The pathways form in about a microsecond and seal in seconds to minutes. The duration of the electric field is tens of microseconds to tens of milliseconds.

The use of electroporation was described by Neumann in the early 1980. The routine use of electroporation became very popular with researchers through the 1980s because it was found to be a practical way to place drugs, or other molecules into cells. In the late 1980s, scientists began to use electroporation for applications in multi-cellular tissue.

In the early 1990s Lluis Mir of the Institute Gustave-Roussy was the first to use electroporation in a human trial to treat external tumors.

Research has shown that the induction of pathways is affected by three major factors. First, cell-to-cell biological variability causes some cells to be more sensitive to electroporation than other cells. Second, for pathways to be induced, the product of the pulse amplitude and the pulse duration has to be above a lower limit threshold. Third, the number of pathways and effective pathway diameter increases with the product of "amplitude" and "duration." Although other factors are involved, this threshold is now understood to be largely dependent on a fourth factor, the reciprocal of cell size. If the upper limit threshold is reached pore diameter and total pore area are too large for the cell to repair by any spontaneous or biological process. The result is irreversible damage to the cell or cell lysis. Because the mechanism of electroporation is not well understood, the development of protocols for a particular application has usually been achieved empirically, by adjusting pulse parameters (amplitude, duration, number, and inter-pulse interval).
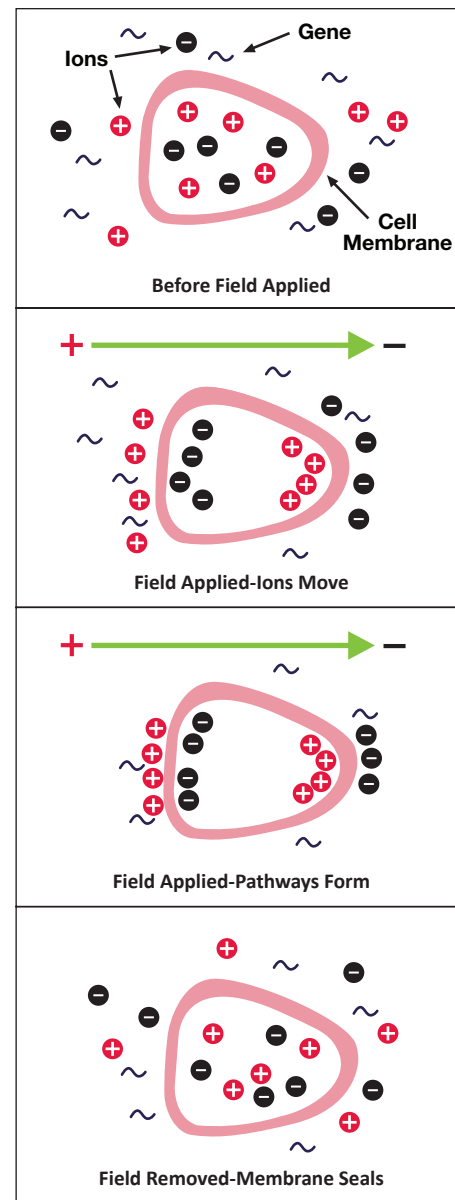

Figure 22: Electroporation

AgilePulse™ IM Tutorial

Research in the late 1980s and early 1990s showed that certain experimental conditions and parameters of electrical pulses may be capable of causing many more molecules to move per unit time than simple diffusion. There is also good evidence (Sukharev et al., 1992) that DNA movement is in the opposite direction of the arrow in the sidebar.

An additional important consideration is when the voltage pulse is applied to the cells and medium that the amount of current that flows is dependent on the conductivity of the material in which the cells are located. Some material is quite conductive and severe heating will occur if the pulse duration is too long. Therefore long duration fields will kill cells by destroying the membrane and heating.

The electric field in which the cells are located is produced by two system components. The first is the voltage waveform generator and the second is the electrode which converts the voltage into the electric field.

Neumann, Sowers and Jordan, 1998, pages 68-73 provides the equation that relates the transmembrane voltage (TMV) to electric field intensity. As the charge accumulates at the membrane, which is a capacitance, the voltage across the membrane increases.

$$voltage \ = \ \frac{charge}{capacitance}$$

As the voltage increases from its quiescent value of a few tenths of a volt to more than 0.5 volts, pathways begin to form. The TMV is given by:

$$TMV = -\tfrac{3}{2} \, E \, r \, / \cos \alpha|$$

where:

E = electric field intensity in volts/cm

r = the cell radius in cm

α = angle off the center line

To produce a TMV of 1 volt across the membrane of a cell with 7 μm radius, the required electric field intensity is:

$$E \ = \ -\tfrac{2}{3} \ \frac{1}{7 \,{*}\,10^{-4}} \ = \ 950 \ volts \, / \, cm$$
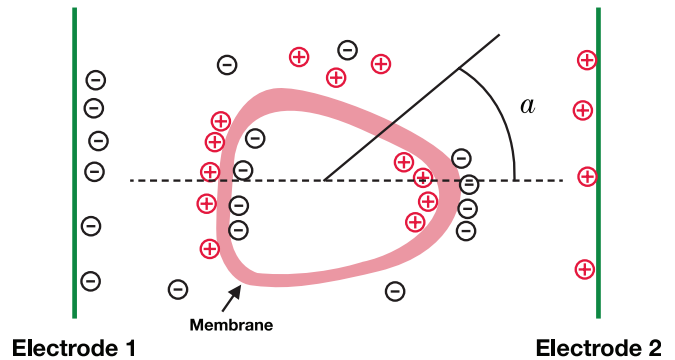

Figure 23: Transmembrane Voltage

Electrode 1   Membrane   Electrode 2

AgilePulse™ IM Tutorial

AgilePulse™ IM Electroporation

In pages 17 to 19 general concepts of electroporation were presented. This section presents the types of waveforms and electrodes available for use in the AgilePulse™ IM system.

Waveforms

Traditional in vivo electroporation uses four to six rectangular pulses that are 100 microseconds in duration at a rate of one per second. Thus the total treatment time is four to six seconds. More advanced waveforms such as the PulseAgile® have been found to be more effective in delivering DNA (Roos et al, 2006, Vertuani et al 2009, Bråve et al 2009). A PulseAgile® waveform consists of various pulse groups with different characteristics from group to group. For example, the study by Dr. Roos found the optimum waveform for induction of high gene expression:

Species	Electric Field V/cm	Pulse Width microseconds	Number	Frequency Hertz	Reference
Equine	1300	100	8	1	Rols, 2002
Canine	1300 (800 if mucosal)	50	8 biphasic	–	Aminkov, 2004
Rodent	1000 to 1300	100	4-8	1 or 5000	Sersa, 2006
Canine	1300	50	8 biphasic	1	Spugnini, 2006
Companion Animal	1300 (800 if oral)	50	8 biphasic	1000	Spugnini, 2003

Group	Pulse Amplitude	Pulse Width	Pulse Interval	Group Interval	Pulse Number
1	450 V (1125 V/cm)	0.05 ms	300 ms	500 ms	2
2	110 V (275 V/cm)	10 ms	300 ms	500 ms	8

The total treatment time for this waveform is 2.98 seconds.

Another more recent study by Roos et al (Mol. Ther., 2009) developed a variant of the PulseAgile® waveform described above. This waveform is called Fast PulseAgile® (PAfast) and differs only in the length of the pulse intervals:

Group	Pulse Amplitude	Pulse Width	Pulse Interval	Group Interval	Pulse Number
1	450 V (1125 V/cm)	0.05 ms	0.2 ms	50 ms	2
2	110 V (275 V/cm)	10 ms	20 ms	50 ms	8

The total treatment time for this waveform is 0.27 seconds. When the pulses are delivered in less than half a second there is only one muscle contraction and tolerability is highly improved.

The AgilePulse™ system has the following waveform settings available:

Pulse Amplitude	Pulse Width	Pulse Interval*	Maximum Duty Cycle	Number of Pulses
50 to 300 V	0.050 to 10 ms	0.2 to 1000 ms	50%	10
310 to 1000 V	0.050 to 1 ms	0.2 to 1000 ms	50%	10

*Limited to 50% Duty Cycle, i.e., (Pulse Width/Pulse Interval) less than or equal to 0.5

Three groups may be used. A group is 1 to 10 pulses with the same parameters. The system software automatically concatenates each group.

AgilePulse™ IM Tutorial

Electrode

Electrodes used in ex vivo and in vivo electroporation are quite different. In ex vivo electroporation, the cells are generally placed in an aqueous ionic medium in a chamber that has parallel plate metal electrodes. This configuration produces very uniform electric fields. For in vivo electroporation the electric fields must be established on a tissue in the body. In this configuration parallel plates are not practical for vaccine delivery.

For in vivo use two parallel rows of needles are used. This configuration was first published in an abstract form by Dr. Julie Gehl in 1997 (Herlev Hospital, Denmark).

Parallel row arrays must be carefully designed to produce electric field intensities as close to uniform as possible to ensure as many cells as possible are exposed to the same field. The key parameters are:

- **Number of needles in each row**
- **Diameter of the needles**
- **Spacing of the needles in the row**
- **Space between the two rows**

In general the more needles per row and the greater the spacing between the rows, the closer the electric field approaches that of a parallel plate. As an example, the calculated electric field for two needles per row is presented below:
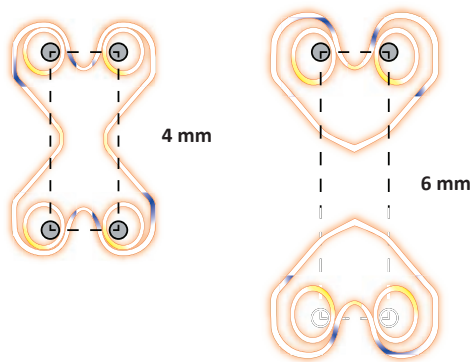

4 mm

6 mm

Figure 24: Two Needles per Row Field

As the spacing between the rows increases the electric field rapidly falls off and cold spots form pores in the cells and transfectant delivery does not occur.

The AgilePulse needle electrodes are specifically designed to produce near uniform electric fields in the treatment volume.

AgilePulse™ IM Tutorial

Needle Array Selection Guide

Item #	Spacing Between Rows	Needles/ row	Needle Diameter	Needle Length	Suggested Use
47-0043	4 mm	4	0.3 mm	3 mm	Small animal, Tibialis muscle
47-0045	4 mm	4	0.3 mm	5 mm	Small animal, Quadriceps muscle
47-0070	6 mm	6	0.7 mm	10 mm	Large animal, Tibialis
47-0080	6 mm	6	0.7 mm	25 mm	Large Animal, Quadriceps
47-0086	6 mm	6	0.7 mm	16 mm	Large animal, Quadriceps
45-0121 (requires 47-0090 or 47-0091 adapter and 45-0206 handle)	5 mm	1	0.3 mm	20 mm	Small or large animal, Tibialis muscle or Quadriceps muscle

The electric field coverage is presented in Figure 25.
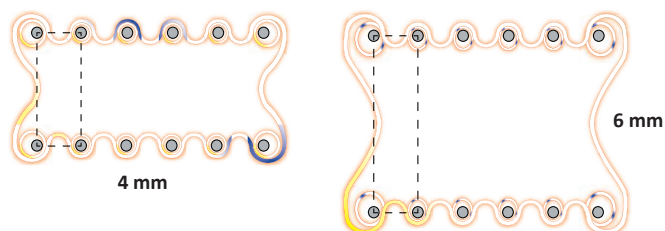

4 mm

6 mm

Figure 25: IMA-4-6-2 and IMA-6-6-2 electric field coverage

There is a significant tradeoff between wider row spacing and larger delivery volume and pulse amplitude. As pulse amplitude increases so does pain of the applied pulse voltage.

AgilePulse™ IM Tutorial

AgilePulse™ In Vivo/Ex Plant Electroporation Protocol

Tissue or Target: Animal Muscle

Transfectant: Plasmid DNA, mRNA, or Protein, or other desired molecule of interest

Electrode Accessory: Please see page 22 for needle array selection guide by animal size and muscle type

Electroporation settings for 4 mm gap electrodes (small animal)					
	Group	Voltage	Pulse Length	Pulse Interval	No. Pulses
Voltage Groups	1	450 V	0.05 ms	0.2 ms	1
	2	450 V	0.05 ms	50 ms	1
	3	110 V	10 ms	20 ms	8
Desired Field Strength: 1125 volts/cm					

Electroporation settings for 5 mm gap electrodes (small animal, large animal)					
	Group	Voltage	Pulse Length	Pulse Interval	No. Pulses
Voltage Groups	1	560 V	0.05 ms	0.2 ms	1
	2	560 V	0.05 ms	50 ms	1
	3	140 V	10 ms	20 ms	8
Desired Field Strength: 1125 volts/cm					

Electroporation settings for 6 mm gap electrodes (large animal)					
	Group	Voltage	Pulse Length	Pulse Interval	No. Pulses
Voltage Groups	1	680 V	0.05 ms	0.2 ms	1
	2	680 V	0.05 ms	50 ms	1
	3	170 V	10 ms	20 ms	8
Desired Field Strength: 1125 volts/cm					

Note: These are recommended electroporation settings. Reducing the pulse interval time below 50 ms in group 2, can lead to error 19. The interval time can be increased to 300 ms or more, if the error recurs. Please contact BTX technical support if an error persists.

AgilePulse Set Up

- Plug-in the needle array handle cable to the AgilePulse generator.

- Turn on AgilePulse™ system.

- Program the appropriate protocol for your electrode as outlined in tables above.

- Input animal ID and Transfectant ID information on AgilePulse instrument.

- Insert needle array electrode tip into needle array handle. Inspect the needle array electrode to ensure that the electrode needles are not bent and all are the same length.

- Optional: For hands-free operation, connect foot pedal to one of the USB ports on the AgilePulse generator.

- Optional: Connect USB flash drive to one of the USB ports on the AgilePulse generator for copy of log data on flash drive. Log data is also stored on Internal Memory on AgilePulse generator automatically.

AgilePulse™ IM Tutorial

Injection

- Prepare transfectant and adjuvant if desired in 0.25 to 1X PBS. Suggested plasmid DNA concentration range is 1 to 4 µg/ml (recommended starting concentration is 2 µg/ml).

- To aid in accurate administration of the procedure and minimize the potential for startle reflex, anesthetize animal as per veterinarian recommendations. The subject should be anesthetized to a level where it is not responsive to a strong toe pinch or other equivalent stimulus.

- Clip hair or depilate to expose target muscle.

- Clean site with 70% alcohol and remove excess alcohol with gauze.

- Anesthetized animal should be oriented to allow for easy exposure of target tissue.

- Obtain tuberculin syringe(s), 3/10 cc (BD model 328431), loaded with transfectant mixture. Use a 30 gauge needle fitted with P10 tubing to ensure an even depth of insertion into the muscle targeted.

- Insert needle tip into the muscle and slowly inject DNA into site.

- Inject 20 to 30 µl DNA solution intramuscularly.

- Repeat the procedure on the other sites if necessary.

Electroporation

- Insert needle electrode so the intramuscular injection is placed between the two needle rows. Begin the process by pressing the "Next" button. This will take you to a screen with a series of buttons.

- Press the "Load" button. Read the "load reading" on the right hand side of the screen. If it is below 100 ohms, then the electrode is inserted far enough. If above 1500 ohms, insert the needles deeper in the tissue.

- After correct insertion has been verified, sequentially press the remaining buttons as they fill-in to start electroporation sequence.

- Keep pushing electrode firmly against animal until pulsing stops.

- Remove electrode after delivery of pulses.

- Check the log file to verify proper delivery.

- Repeat electroporation as needed, then press "Done" icon to return to the main screen.

- Observe animal and keep warm until it recovers from anesthesia. Put animal back into its cage.

Specifications

AgilePulse™ IM	
Pulsing	
Resistance Pulsing	4 µs at 5 volts every second
Pulse Protocol Parameters (Parameters in a Group)	
Pulse Width	50 µs to 1 ms 50 to 1000 volts 50 µs to 10 ms 50 to 300 volts
Pulse Current Trip	26 amp
Load Range	80 to 1000 ohms Recommended for low resistance tissue such as quadriceps muscle. • Small animal: resistance range 200 to 400 ohms • Large animal: resistance range 80 to 250 ohms
Number of Pulses	1 to 10
Maximum Duty Cycle	50%
Interval	200 µs to 1 sec (pulse start to pulse start)
Number of Groups	3
Pulse Measurement (Internal Digitizer)	
Levels	12 bit
Samples	Pulse width/8 minimum 100 µs
Data Stored Internally and on External USB Key*	
Raw Data	DV<Date>.xml
Log Data	DV<Date>.txt
CSV Data	DV<Date>.csv
Electrical and Mechanical	
Electrode Connector	Fischer Series 4032
Current Ratings	5A
Input Voltage frequency Ratings	100 to 240 VAC, 50/60 Hz
Dimensions (W x H x L)	30.5 cm x 16.5 cm x 36.8 cm (12 in x 6.5 in x 14.5 in)
Weight	5.7 kg (12.5 lb)
Warranty	2 years
Operating Temperature	4°C to 40°C (40°F to 104°F)
Storage Temperature	-10°C to 70°C (14°F to 158°F)
Operating Humidity	20% to 80% RH, non-condensing
Storage Humidity	20% to 80% RH, non-condensing
Mode of Operation	Continuous
Classification	Class I
Ingress	IP2X
Installation	Category II

Other Specifications	
Supplier Name	BTX
Supplier Address	84 October Hill Rd., Holliston, MA 01746
Supplier Phone Number	508-893-8999
Regulatory Certifications	CE, ETL (UL, CSA), FCC, WEEE, EU and RoHS

*All data automatically stored in internal memory and may be downloaded to an external USB Key.
Maximum Data Logs stored and retrievable from internal flash memory >20,000.

Maintenance

The AgilePulse system requires no special maintenance other than keeping it clean.

If the surface of the touch screen display or enclosure needs to be cleaned, use a standard (non-ammonia) glass cleaner or mild detergent with warm water and a soft, lint free paper or cloth towel. Do not apply the cleaning solution directly to the screen or enclosure, to avoid liquid running into other parts of the cabinet. Put a small amount of cleaner on the towel and gently rub the screen or enclosure. Avoid hard rubbing, abrasives, or harsh solvents like alcohol or ammonia.

Fuse Replacement

1. Make sure the power cord is disconnected from the main supply before servicing the fuse.

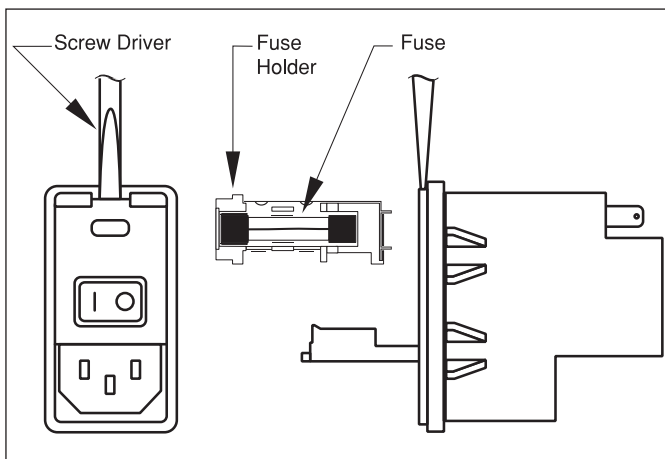2. Replace fuse with (240V/5A slo-blo) 5 x 20 mm.

Figure 26: Fuse Replacement

Electrode Care and Cleaning

Needle Array electrodes are disposable arrays made of medical grade plastic with surgical steel needles. However needle arrays can be used up to 500 pulses if cleaned immeadiately following use.

Post Electroporation Electrode Care

- Rinse electrode contact area in warm water.

- Wash with dilute detergent (1% V/V). Needles may be brushed gently with a soft brush (for example toothbrush).

- Rinse well with lab grade deionized water.

- Air dry.

- Check for bent or depressed needles; all needles should be equal length.

- Discard any needle arrays with bent or uneven length needles.

Electrode Sterilization

- Soak needle end of electrode for 10 minutes in 4% Sodium Hydroxide (suggested use vessel such as a petri dish).

- Rinse well with sterile lab grade water.

- Spray with 70% alcohol.

- Air dry.

- Replace needle protection cap for storage until next use.

Ordering Information

Item No.	Description	Qty.
Electroporation Systems		
47-0500N	AgilePulse In Vivo IM (Intra-Muscular) System	1 Ea
47-0501N	AgilePulse In Vivo IM (Intra-Muscular) Generator Only	1 Ea
Needle Array Electrodes		
47-0043	4-Needle Array, 4 mm gap, 3 mm length	1 Ea
47-0045	4-Needle Array, 4 mm gap, 5 mm length	1 Ea
47-0070	6-Needle Array, 6 mm gap, 10 mm length	1 Ea
47-0080	6-Needle Array, 6 mm gap, 25 mm length	1 Ea
47-0086	6-Needle Array, 6 mm gap, 16 mm length	1 Ea
45-0168	2-Needle Array Kit, 5 mm gap, Pkg of 6, with Needle Array Handle (requires 47-0090 or 47-0091 adapter)	1 Kit
45-0121	2-Needle Array electrodes, 5 mm gap, Pkg of 6 (requires 47-0090 or 47-0091 adapter and 45-0206 handle)	1 Pkg

Item No.	Description	Qty.
Accessories		
47-0420	AgilePulse Foot pedal	1 Ea
47-0090	AgilePulse In Vivo Adapter box, for 4 mm banana cable electrodes (2 Pin Connector). For generators with Serial No. AP-5030-IM or earlier.	1 Ea
47-0000	AgilePulse in Vivo Needle Array Handle (2 Pin Connector). For generators with Serial No. AP-5030-IM or earlier.	1 Ea
45-0206	Array Handle, for 5 mm gap, 2-Needle Array	1 Ea
47-0091	AgilePulse In Vivo Adapter box, for 4 mm banana cable electrodes (3 Pin Connector). For generators with Serial No. AP-5031-IM or later. 1 Ea	
47-0092	AgilePulse In Vivo Needle Array Handle, for 4 mm banana cable electrodes (3 Pin Connector). For generators with Serial No. AP-5031-IM or later. 1 Ea	
Service Offerings		
45-9996	AgilePulse 1 Year Extended Warranty	1 Ea
45-0909	AgilePulse System Validation Certificate	1 Ea

References

Anderson AM, Baranowska-Hustad M, Braathen R, Grodeland G, Bogen B. Simultaneous Targeting of Multiple Hemagglutinins to APCs for Induction of Broad Immunity against Influenza. *J Immunol.* 2018 Mar 15;200(6):2057–2066.

Angamuthu M, Murthy SN. Therapeutic Applications of Electroporation. In: Dragicevic N, Maibach H, eds. *Percutaneous Penetration Enhancers Physical Methods in Penetration Enhancement.* Berlin, Heidelberg: Springer;2017:122-137.

Bartoletti DC, Harrison GI. and Weaver JC. The number of molecules taken up by electroporated cells: quantitative determination. *FEBS Lett.* 1989:256:4-10.

Biragyn A, et al. Tumor-associated embryonic antigen-expressing vaccines that target CCR6 elicit potent CD8+ T cell-mediated protective and therapeutic antitumor immunity. *J Immunol.* 2007 Jul 15;179(2):1381-1388.

Bongard N, Lapuente D, Windmann S, Dittmer U, Tenbusch M, Bayer, W. Interference of Retroviral Envelope with Vaccine-Induced CD8+ T Cell Responses is Relieved by Co-Administration of Cytokine-Encoding Vectors. *Retrovirology.* 2017;14: 28.

Bråve A, et al. Late administration of plasmid DNA by intradermal electroporation efficiently boosts DNA-primed T and B cell responses to carcinoembryonic antigen. *Vaccine.* 2009 Jun 8;27(28): 3692-3696.

Chang, DC, Chassy BM, Saunders JA and Sowers AE, eds. Guide to Electroporation and Electrofusion. San Diego: Academic Press;1992.

Christopher W, et al, Long-term multilineage engraftment of autologous genome-edited hematopoietic stem cells in nonhuman primates. *Blood.* 2016;127:2416-2426.

Daftarian MP, Vosoughi A, Lemmon V. Gene-Based Vaccination and Screening Methods to Develop Monoclonal Antibodies. *Methods Mol Biol.* 2014;1121:337-346.

Davtyan H, et al. BTX AgilePulse(TM) System is an Effective Electroporation Device for Intramuscular and Intradermal Delivery of DNA Vaccine. *Curr Gene Ther.* 2014;14(3):190-199.

Denies S, Cicchelero L, Polis I, Sanders NN. Immunogenicity and safety of xenogeneic vascular endothelial growth factor receptor-2 DNA vaccination in mice and dogs. *Oncotarget.* 2016;7(10):10905-10916.

Denies S, et al. Evaluation of a Xenogeneic Vascular Endothelial Growth Factor-2 vaccine in Two Preclinical Metastatic Tumor Models in Mice. *Cancer Immunol Immunother.* 2017 Dec;66(12):1545-1555.

Dimitrov DS, and Sowers AE. Membrane electroporation — fast molecular exchange by electroosmosis. *Biochimica et Biophysica Acta.* 1990 1022:381-392.

Djuzenova CS, Zimmermann U, Frank H, Sukhorukov VL, Richter E, Fuhr G. Effect of medium conductivity and composition on the uptake of propidium iodide into electropermeabilized myeloma cells. *Biochimica et Biophysica Acta.* 1996;1284:143-152.

Disalvo EA and Simon SA, eds. Permeability and Stability of Lipid Bilayers. Boca Raton: CRC Press;1995.

Eyquem J, et al. Targeting a CAR to the TRAC Locus with CRISPR/Cas9 Enhances Tumour Rejection. *Nature.* 2017;543:113-117.

Gehl J. "Electroporation for Drug and Gene Delivery in the Clinic: Doctors Go Electric." *Electroporation Protocols.* Humana Press; 2008. 351-359.

Grodeland G, Fredriksen AB, Løset GÅ, Vikse E, Fugger L, Bogen B. Antigen Targeting to Human HLA Class II Molecules Increases Efficacy of DNA Vaccination. *J Immunol.* 2016;197(9):3575-3585.

Hawksworth DJ, Ziemann R, Manoj S. Enhanced Immune Response Following DNA Immunization Through In Vivo Electroporation. *Monoclon Antib IImmunodiagn Immunother.* 2016 Jul 27;35(5):239-244.

Johansson DX, Ljungberg K, Kakoulidou M, Liljestrom P. Intradermal Electroporation of Naked Replicon RNA Elicits Strong Immune Responses. *PLoS One*, 2012 Jan 4;7(1):e29732.

Juillerat A, et al. An Oxygen Sensitive Self-Decision Making Engineered CAR T-Cell. *Sci Rep.* 2017 Jan 20;7:39833.

Juillerat A, et al. Design of Chimeric Antigen Receptors with Integrated Controllable Transient Functions. *Sci Rep.* 2016 Jan 11;6:18950.

References

Klenchin VA, Sukharev SM, Chernomordik LV, Chizmadzhev YA, Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis. *Biophys J.* 1991;60:804-811.

Leyman, B, et al. Comparison of the Expression Kinetics and Immunostimulatory Activity of Replicating mRNA, Nonreplicating mRNA, and pDNA after Intradermal Electroporation in Pigs. *Molecular Pharmaceutics.* 2018:15 (2):377-384.

Lladser A, Ljungberg K, Tufvesson H, Tazzari M, Roos A, Quest FG, Kiessling R. Intradermal electroporation with a survivin DNA vaccine induces CTLs against a self-epitope, suppresses angiogenesis and confers long-term protection against mouse melanoma. *Cancer Immunol Immunother.* 2010 Jan;59(1):81-92.

Lladser A, et al. Intradermal electroporation with a survivin DNA vaccine induces CTLs against a self-epitope, suppresses angiogenesis and confers long-term protection against mouse melanoma. *Cancer Immunol Immunother.* 2010 Jan;59(1):81-92. doi: 10.1007/s00262-009-0725-4. Epub 2009 Jun 14.

Lundberg K, Roos AK, Pavlenko M, Wehrum D, Pisa P. A modified epitope identified for generation and monitoring of PSA-specific T cells in patients on early phases of PSA-based immunotherapeutic protocols. *Vaccine.* 2009 Mar 4;27(10):1557-65.

Neuman E, Sowers AE, and Jordan C.A, eds. Electroporation and Electrofusion in Cell Biology. New York: Plenum Press;1989.

Neuman E, Toensing K, Kakorin S, Budde P, Frey J. Mechanism of electroporative dye uptake by mouse B cells. *Biophys J.* 1998;74:98-108.

Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. *Bioelectrochem Bioenerg.* 1999;48:3-16.

Nickoloff, Jac A., ed. Plant Cell Electroporation and Electrofusion Protocols. *Methods in Molecular Biology*, Volume 55. Totowa, New Jersey:Humana Press;1995.

Peterson CW, et al. Long-Term Multi-Lineage Engraftment of Genome-Edited Hematopoietic Stem Cells after Autologous Transplantation in Nonhuman Primates. *Blood.* 2016 May 19;127(20):2416-2426.

Roos AK, et al. Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment. *PLoS ONE.* 2009 Sep 30;4(9):e7226.

Roos AK, King A, Pisa P. DNA vaccination for prostate cancer. *Methods Mol Biol.* 2008;423:463-72.

Roos AK, Moreno S, Leder C, Pavlenko M, King A, Pisa P. Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation. *Mol Ther.* 2006;13:320-327.

Roos AK, Eriksson F, Walters D, Pisa P, King A. Optimization of skin electroporation in mice to increase tolerability of DNA vaccine delivery to patients. *Mol Ther.* 2009 Sep;17(9):1637-42.

Shixia Wang, Shan Lu. DNA Immunization. *Curr Protoc Microbiol.* 2013;31:18.3.1-18.3.24.

Sukharev SI, Klenchin VA, Serov SM, Chernomordik LV, Chizmadzhev Y. Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores. *Biophys J.* 1992;63:1320-1327.

van der Woning B, et al. DNA immunization combined with scFv phage display identifies antagonistic GCGR specific antibodies and reveals new epitopes on the small extracellular loops. *mAbs.* 2016:8(6):1126-1135.

Vertuani S, et al. HER-2/neu mediated down-regulation of MHC class I antigen processing prevents CTL-mediated tumor recognition upon DNA vaccination in HLA-A2 transgenic mice. *Cancer Immunol Immunother.* 2009 May;58(5):653-664. Epub 2008 Sep 27.

Wang L, et al. Evaluation of Candidate Vaccine Approaches for MERS-CoV. Nat Comm. 2015 Jul28;6:7712.

Wolf H, Rols M P, Boldt E, Neumann E, Teissie J. Control by pulse parameters of electric field mediated gene transfer in mammalian cells. *Biophys J.* 1994;66:524-531.

Zerbib D, Amalric F, Teissie J. Electric field mediated transformation: isolation and characterization of a TK+ subclone. *Biochem Biophys Res Commun.* 1985;129:611-618.

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