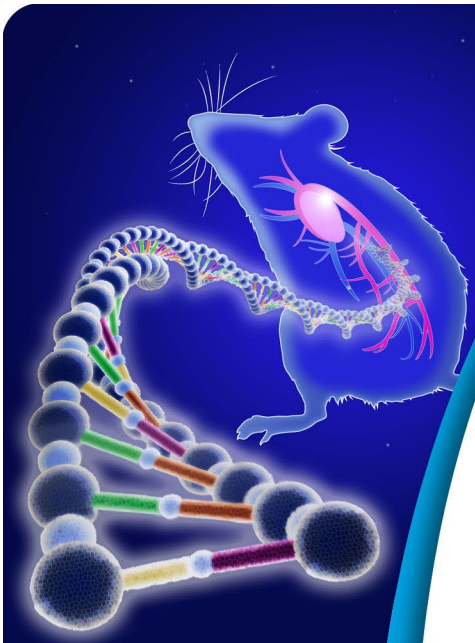

AgilePulse™ ID

In Vivo Gene Delivery System

USER'S MANUAL

BTX®

a division of **Harvard Bioscience, Inc.**

AgilePulse™ ID *In Vivo* Gene Delivery System

RESEARCH ONLY

BTX Harvard Apparatus
84 October Hill Rd
Holliston, MA 01746, USA

phone 1-508-893-8999 • fax 1-800-429-5732 • www.btxonline.com

BTX Warranty

Harvard Apparatus warranties the AgilePulse™ In Vivo Gene Delivery System 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 AgilePulse™ instrument 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 electrodes not manufactured by BTX - HARVARD APPARATUS are used with the AgilePulse instrument. 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.

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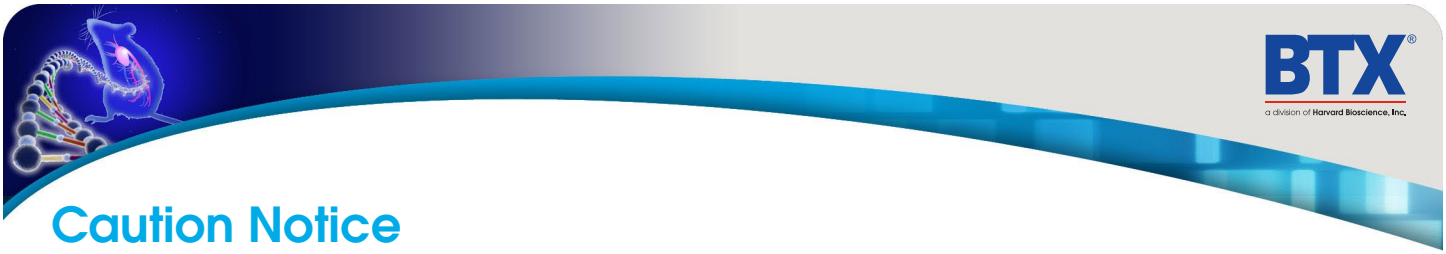


Caution Notice

This instrument contains a high voltage power supply adjustable to 1,000 volts. 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 instrument is placed into operation.**
- **Removing the cover may 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**
- **If a problem occurs during a run, push the STOP/RESET button on the front panel.**

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

1 Introduction

The AgilePulse™ IM system was developed to deliver small molecules using pulsed electric fields (electroporation). The system has been designed for research purposes.

The system has three components: the AgilePulse Waveform Generator, the Intramuscular Array (IMA) electrodes (4 & 6) parallel needle arrays and the User Manual. Using other components may damage the system, will certainly provide degraded performance and invalidate the warranty.

NOTE: The AgilePulse Waveform Generator contains a high voltage power supply and was designed with safety features to protect the user and the equipment. If used properly, the AgilePulse Waveform Generator is a safe and reliable instrument.

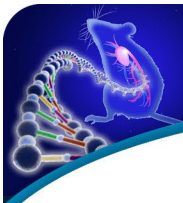
Front and Back Panel Symbols

Caution Risk of Electric Shock | **Operating Instructions** | **Caution** | **AC Mains** | **Off** | **On** | **Fuse**

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

For research only. Not for clinical use on patients.



2 Items of Particular Interest

The AgilePulse™ ID DNA Vaccine Delivery System is a sophisticated system designed for delivery of DNA vaccines to the skin. The following are important:

1. **The system shall only be used as described in this User Manual, the user is required to read the User Manual and undergo training before use.**
2. **Certain concepts and processes are covered by patents, patents pending, know-how and trade secrets.**
3. **The IDA electrode tip is disposable, single use , and sterile unless otherwise indicated on the wrapper label.**
4. **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.**
5. **Do not connect or disconnect any electrode from the waveform generator when the pulse waveforms are being applied.**
6. **Do not put your fingers or any other object on the ID electrode tip while pulse waveforms are being applied.**
7. **This system produces voltages as high as 1000 volts and may cause injury or death if used improperly.**
8. **The system shall not be used in the presence of flammable anesthetic.**

BTX welcomes any comments from the users to improve this manual.

3 AgilePulse™ Tutorial

3.1 Applications

The AgilePulse™ system is used to deliver DNA vaccines to study the effects and potential of this application for research purposes only. The AgilePulse™ system has been shown to increase DNA delivery to cells resulting in 100-1000 times higher protein expression compared to hypodermic needle injection alone (Roos et al., 2006).

The AgilePulse™ system is typically used to deliver cancer vaccines in doses of 50 μg or more. The AgilePulse™ system includes a AgilePulse voltage waveform generator and an Intradermal Array (IDA) electrode used to convert the voltage to electric fields which deliver the vaccine directly into cells in the skin.

3.2 DNA Vaccine Delivery Methods

There are several methods used to deliver DNA vaccines.

System Name	Company	Mechanism	Target	Factors (all pain ratings are estimates)
AgilePulse™	BTX	Inject vaccine in dermis with hypodermic needle, insert needle array, apply electric field to deliver into cells	Dermis	• 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
Many (muscle electroporation)	Many	Inject into muscle insert electrode and apply electric field	Muscle	• Large dose • Significant pain • Pain 5-6 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

3.3 DNA Delivery Into Cells Using Electroporation

3.3.1 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 1990's 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).

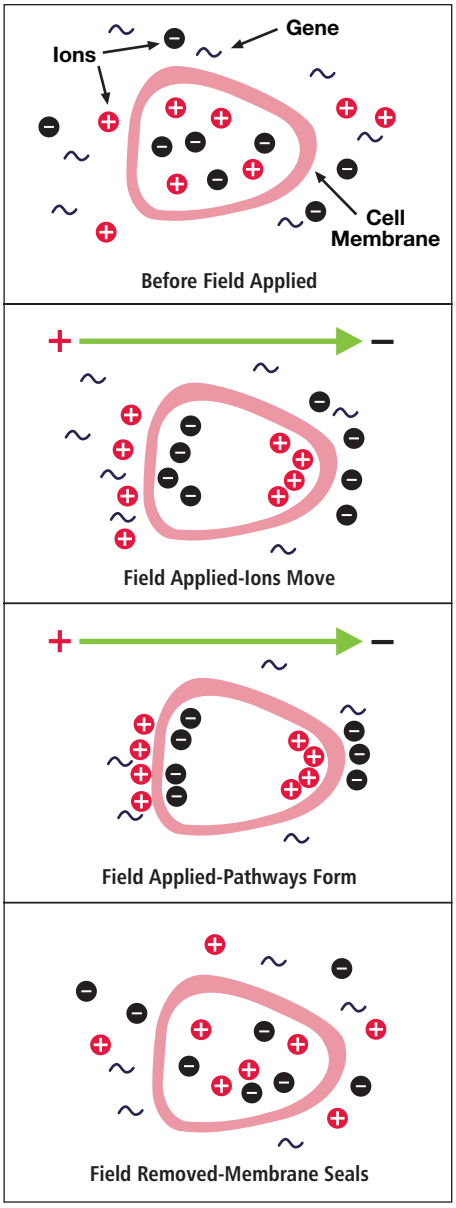


Figure 3-1: Electroporation

3.3 DNA Delivery Into Cells Using Electroporation (cont'd)

3.3.1 General Electroporation Discussion (cont'd)

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.

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

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:

$$TMV \approx \tfrac{3}{2} E\, r\, |\cos \alpha|$$

where:

E = electric field intensity in volts/cm

r = the cell radius in cm

α = angle off the center line

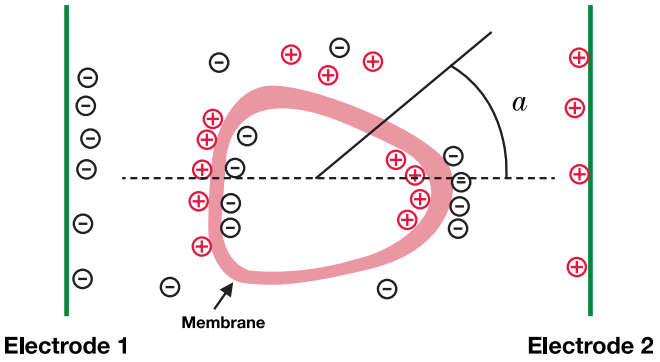


Figure 3-2: Transmembrane Voltage

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

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

General References:

Dimitrov, D.S., and Sowers, A.E., (1990) Membrane electroporation - fast molecular exchange by electroosmosis. Biochimica et Biophysica Acta 1022: 381-392.

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

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

E. A. Disalvo and S.A. Simon, eds. (1995) Permeability and Stability of Lipid Bilayers (CRC Press, Boca Raton), p 105-121.

Chang, D.C., Chassy, B.M., Saunders, J.A. and Sowers, A.E., eds. (1992) Guide to Electroporation and Electrofusion, (Academic press, San Diego), 581 pp.

Neuman, E., Sowers, A.E., and Jordan, C.A.., eds. (1989) Electroporation and Electrofusion in Cell Biology, (Plenum Press, New York) 581 pp.

Bartoletti, D. C., Harrison, G. I., & Weaver, J. C. (1989). The number of molecules taken up by electroporated cells: quantitative determination. FEBS Lett., 256, 4-10.

Djuzenova, C. S., Zimmermann, U., Frank, H., Sukhorukov, V. L., Richter, E., & Fuhr, G. (1996). Effect of medium conductivity and composition on the uptake of propidium iodide into electropermeabilized myeloma cells. Biochim.Biophys.Acta, 1284, 143-152.

Klenchin VA, Sukharev SM, Chernomordik LV, Chizmadzhev YA, Electricaly induced DNA uptake by cells is a fast process involving DNA electrophoresis, 1991, Biophys J. 60:804-811 Neumann, E., Kakorin, S., & Toensing, K. (1999). Fundamentals of electroporative delivery of drugs and genes. Bioelectrochem. Bioenerg., 48, 3-16.

Neuman, E., Toensing, K., Kakorin, S., Budde, P., & Frey, J. (1998). Mechanism of electroporative dye uptake by mouse B cells. Biophys.J., 74, 98-108. Sukharev, S. I., Klenchin, V. A., Serov, S. M., Chernomordik, L. V., & Chizmadzhev, Y. (1992). Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores. Biophys.J., 63, 1320-1327.

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

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

3.3 DNA Delivery Into Cells Using Electroporation (cont'd)

3.3.2 AgilePulse™ Electroporation

In section 3.3.1 general concepts of electroporation were presented. This section presents the types of waveforms and electrodes available for use in the AgilePulse™ 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 eliciting T-cell and antibody responses simultaneously when delivering DNA vaccines (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 and high induction of antigen-specific T cells to be:

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. (Waveforms for clinical use must be optimized in pre-clinical studies.)

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 (this waveform is currently tested in three clinical studies). 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 1-10 pulses with the same parameters. The system software automatically concatenates each group.

3.3 DNA Delivery Into Cells Using Electroporation (cont'd)

3.3.2 AgilePulse™ Electroporation (cont'd)

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 in human 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:

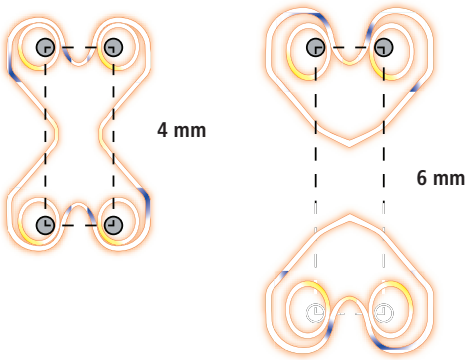


Figure 3-3 Two Needles per Row Field

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

The AgilePulse™ needle electrodes are specifically designed to produce near uniform electric fields in the treatment volume. There are currently three ID electrodes available.

Model No.	Row Space	Needle/ Row	Needle Diameter	Needle Length	Use
47-0040	4 mm	4	0.3 mm	2 mm	Animal Only
47-0050	4 mm	6	0.3 mm	2 mm	Animal Only
47-0060	6 mm	6	0.3 mm	2 mm	Animal Only

The electric field coverage is presented in Figure 3-4.

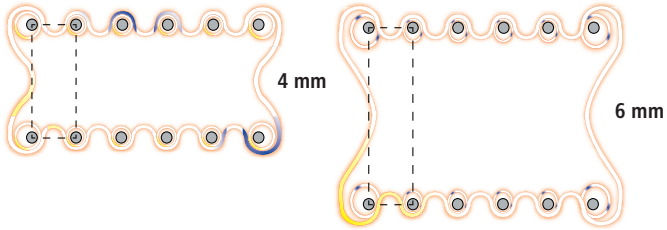


Figure 3-4: IDA-4-6-2 and IDA-6-6-2 electric field coverage

The recommended bleb placement for the IDA-4-6-2 and IDA-6-6-2 electrodes is shown in Figure 3-5.

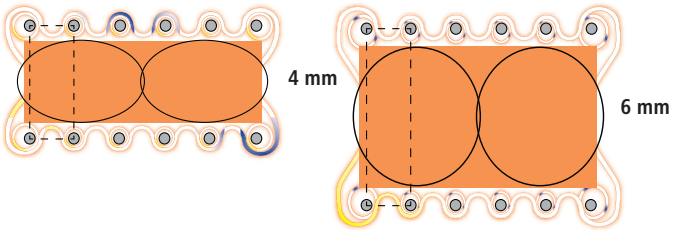


Figure 3-5: Bleb Formation ID-4-6-2 and ID-6-6-2

The total bleb volume for each is approximately:

Model No.	Area	Depth	Volume (approx)	Pulse Voltage V/d= 1000 V/cm	Pulse Voltage V/d= 1500 V/cm
47-0050	8.7 mm^2 x2	2 mm	50-90 μl	400	600
47-0060	15.6 mm^2 x2	2 mm	100 μl	600	900

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.

3.3.3 Specific References

There are ten specific references for this technique:

Gehl, Julie, Thesis, Copenhagen, May 2002

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

Biragyn A, Schiavo R, Olkhanud P, Sumitomo K, King A, McCain M, Indig FE, Almanzar G, Baatar D. 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):1381-8.

Roos A-K, King A, Pisa P. DNA vaccination for prostate cancer. Electroporation protocols: Experimental and Clinical Medicine. Editor S. Li © Humana Press Inc., Totowa, NJ. Methods Mol Biol. 2008;423:463-72.

Vertuani, S, Triulzi, C, Roos, A-K, Pisa, P, Charo, J, Lemonnier, F, Nishimura, M, Seliger, S, Kiessling, R. 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. 2008. Cancer Immunol Immunother. 2009 May;58(5):653-64. Epub 2008 Sep 27.

Lundberg K, Roos A-K, 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.

Bråve A, Hallengärd D, Gudmundsdotter L, Stout R, Walters R, Wahren B, Hallermalm K. 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):3692-6. Epub 2009 May 3.

Roos, A-K, Eriksson, F, Walters, D, Pisa, P, King, A. Optimization of skin electroporation in mice to increase tolerability of DNA vaccine delivery to patients. Molecular Therapy, 2009 Sep;17(9):1637-42. Epub 2009 Jun 16.

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. 2009 Jun 14. [Epub ahead of print]

Roos A-K, Eriksson E, Timmons J, Gerhardt J, Nyman U, Gudmundsdotter L, Bråve A, Wahren B, Pisa P. Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment. PLoS ONE. 2009 Sept 30.

4 AgilePulse™ System Components & Set-Up

4.1 Introduction

The AgilePulse™ system provides the capability of delivering DNA vaccines to the dermis. The system is designed for easy set-up and operation.

Specifications are presented in Appendices A and B, Tabs 7 and 8.

The AgilePulse™ system is composed of three subsystems:

- **Touchscreen controlled AgilePulse Waveform Generator**
- **Intradermal Array (ID) parallel, needle electrodes**
- **User Manual**

Optional equipment available includes:

- **Foot Pedal (47-0420)**

Figure 4-1: Waveform Generator

4.2 The AgilePulse Waveform Generator

The Waveform Generator (Figure 4-1) 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.

4 AgilePulse™ System Components & Set-Up

4.2 The AgilePulse Waveform Generator (cont'd)

4.2.1 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 ID Electrode Connector, and the User Touch Screen (Figure 4-2).

One USB port can be used for a Memory Key. The data logs that result from a vaccination are automatically stored on the Key if one is inserted. The other USB port may be used for the optional Foot Pedal (Appendix C, Tab 9).

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.

Figure 4-2: AgilePulse Front Panel

4.2.2 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.

4.3 Intradermal ARRAY Electrode (IDA)

The IDA 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 DNA vaccine 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.

Available needle configurations are described in Appendix A.

4.4 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.

4.4.1 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 (O). Verify the power cord is properly plugged into the wall. Verify the wall socket is functional. If necessary, you may need to check the fuse on the back panel of the Waveform Generator. If it is faulty, replace it with the exact type fuse (240V/5A, slo-blo) 5x20 mm.

If the power is properly applied, the screen should appear as that shown in Figure 4-3. 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 4-4). If the NEXT button turns RED, then the internal checks failed (Figure 4-5). The system cannot be used if the button is red. Contact BTX Customer Service for assistance.

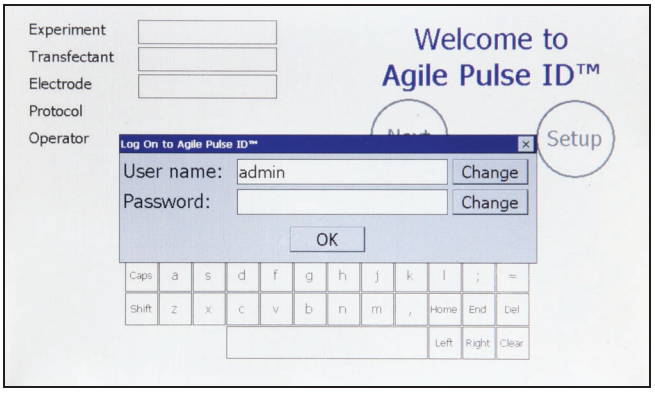


Figure 4-3: Login Screen
(visible about 10 seconds after power on)

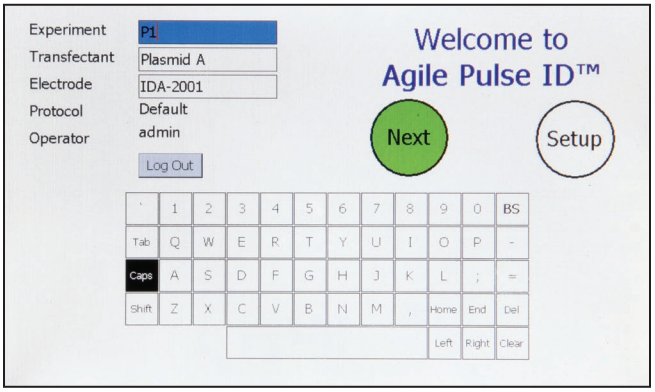


Figure 4-4: System OK Screen (Operating Correctly)

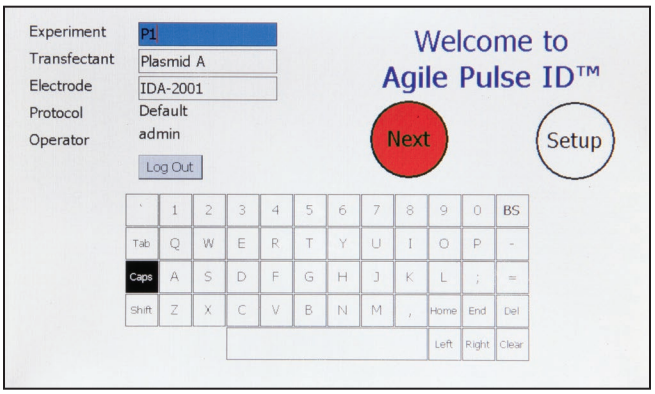


Figure 4-5: Self Check Was Not Successful Screen

4.4 Setting Up the System (cont'd)

4.4.2 Touch Screen Cleaning

If the surface of the touch screen display 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, to avoid liquid running into other parts of the cabinet. Put a small amount of cleaner on the towel and gently rub the screen. Avoid hard rubbing, abrasives, or harsh solvents like alcohol or ammonia.

5 System Operation

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

5.1 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 vaccine 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.

5.1.1 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.

5.2 Intradermal Array (IDA) Electrode

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

5.2.1 IDA 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.

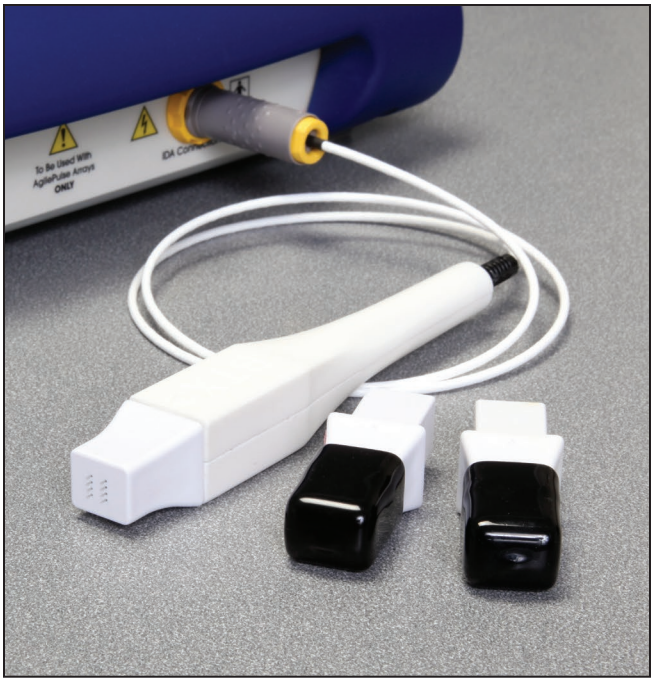


Figure 5-1: IDA Electrode Handle

5.2 Intradermal Array (ID) Electrode (cont'd)

5.2.2 ID Electrode Tip

The IDA tip is recommended for and is single use, disposable. 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 pouch 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.

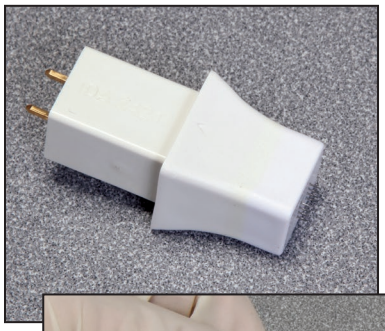


Figure 5-2: IDA Tip

Figure 5-3: Attaching IDA Tip to Handle

Once the vaccine is ready for administration, remove the protective cover from the IDA Tip taking care not to disconnect the tip from the handle.

5.3 Waveform Generator

5.3.1 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.

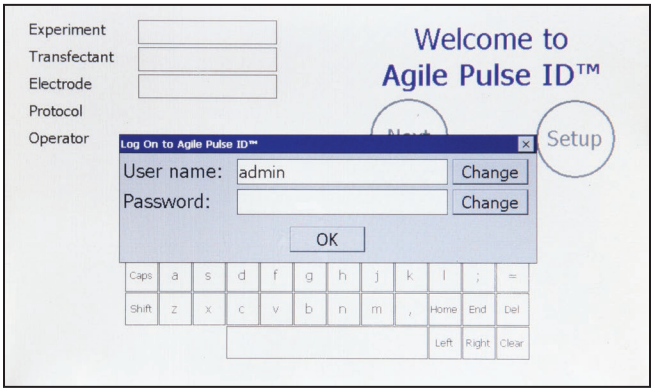


Figure 5-4: User Login Screen

5.3 Waveform Generator (cont'd)

5.3.1 Login Screen – User Identification Input (cont'd)

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™ 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 5-4 on first login. 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 login

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.

5.3.2 Welcome Screen – Identification Input

An opening screen (Figure 5-5) 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 ID, Transfectant and Electrode ID.

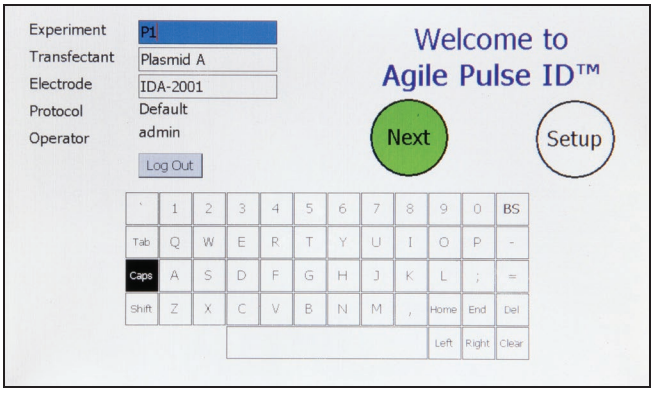


Figure 5-5: Opening Screen

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.

5.3 Waveform Generator (cont'd)

5.3.3 Experiment Delivery Screen Functions and Operation

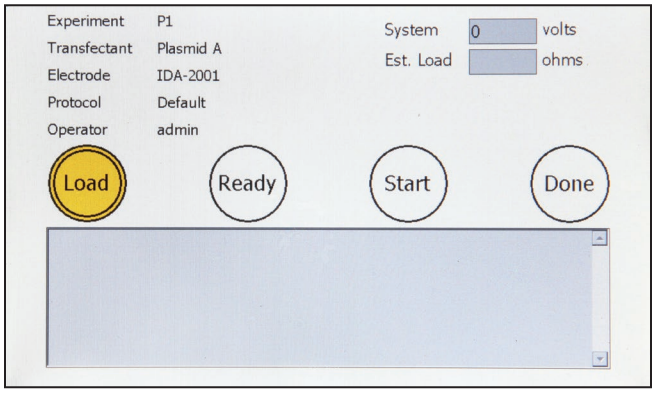


Figure 5-6: Delivery Screen - Initialization

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

1. The researcher injects the vaccine forming a bleb under the skin 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. Est. Load in ohms is displayed. This number is the effective resistance of the animal's skin and will continue to be updated every second.

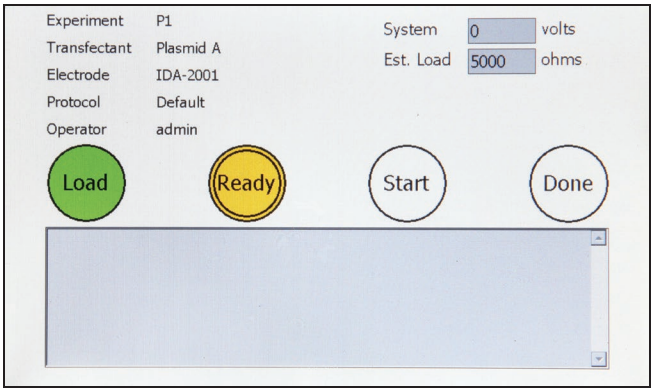


Figure 5-7: Delivery Screen - Load Estimation

3. If the LOAD Reading is within range (Less than 3500 ohms for skin) 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.

5.3 Waveform Generator (cont'd)

5.3.3 Experiment Delivery Screen Functions and Operation (cont'd)

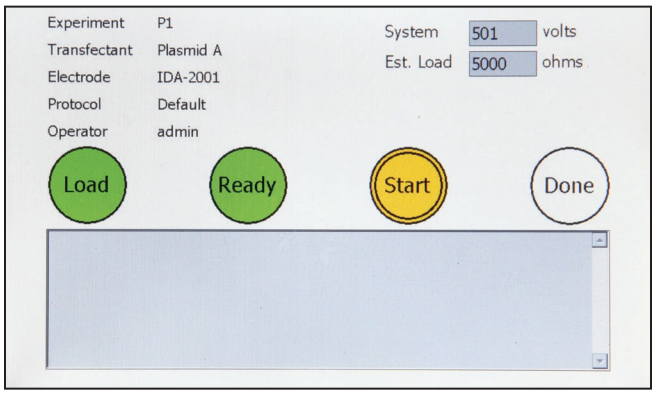


Figure 5-8: Delivery Screen - High Voltage Power Supply Charge

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.

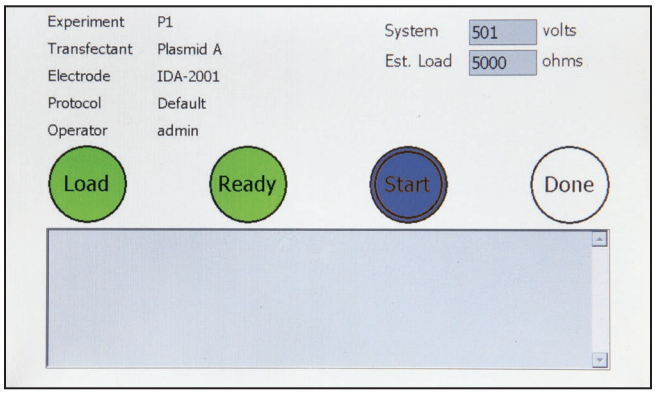


Figure 5-9: Delivery Screen - Pulse Waveform Active

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.

6. All run history log data are automatically stored in internal Flash Memory as well as a USB Key, if one is inserted.

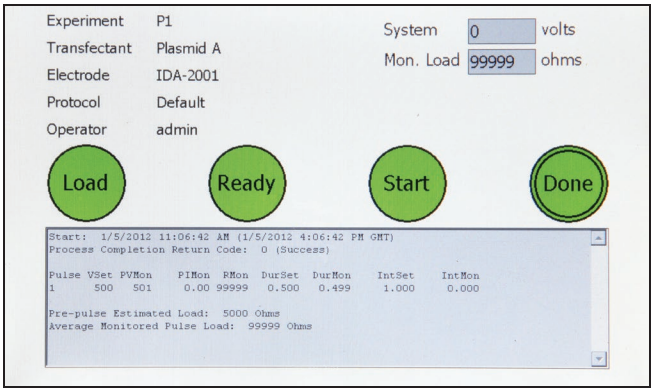


Figure 5-10: Delivery Screen - Shutdown

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

5.3 Waveform Generator (cont'd)

5.3.4 Experiment Delivery Logs

All collected data from the therapeutic/vaccine delivery 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 ID	•	•	•
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	•		

5.3 Waveform Generator (cont'd)

5.3.4 Experiment Delivery Logs (cont'd)

5.3.4.1 Text Log

The text log contains the same information that appears on the vaccine 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 5-11.

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:     IDA-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 5-11: Text Log Screen

5.3 Waveform Generator (cont'd)

5.3.4 Experiment Delivery Logs (cont'd)

5.3.4.2 CVS 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 5-12, 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.

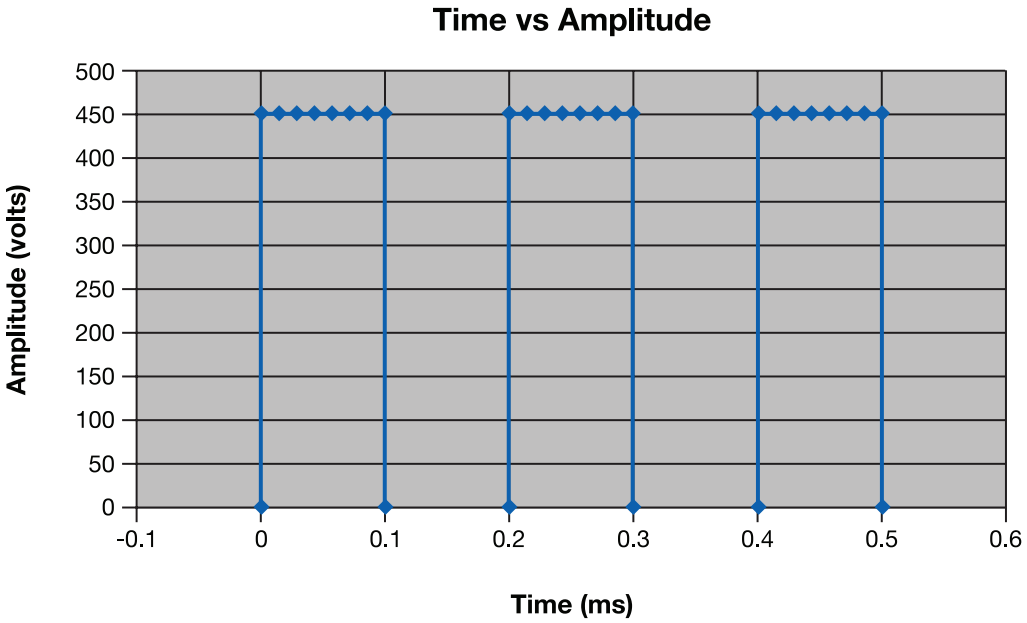


Figure 5-12: Sample Amplitude Analysis

5.3 Waveform Generator (cont'd)

5.3.5 Delivery Completion Return Codes

Return codes are available following system shutdown describing how it took place.

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

5.3 Waveform Generator (cont'd)

5.3.6 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 therapeutic delivery logs. To enter this mode, press the Setup button on the startup screen.

5.3.6.1 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.

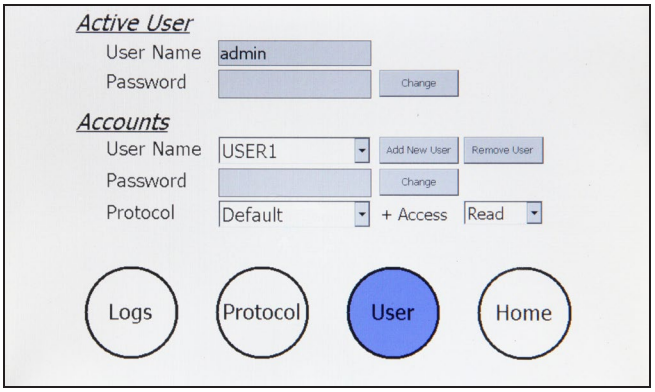


Figure 5-13: User Account Screen

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.

5.3 Waveform Generator (cont'd)

5.3.6 Software Maintenance Mode (cont'd)

5.3.6.2 Log Retrieval

All previous therapeutic 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 vaccine 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.

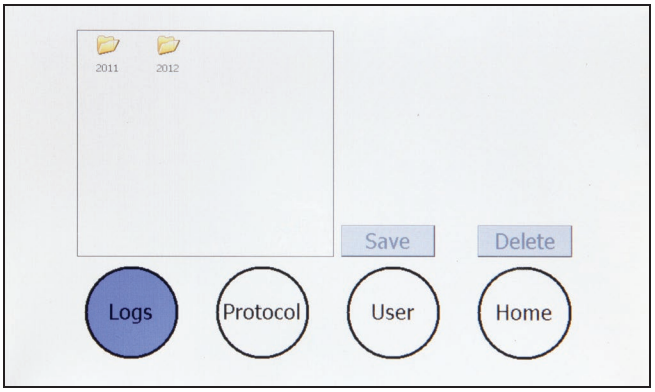


Figure 5-14: Log Retrieval Screen

5.3 Waveform Generator (cont'd)

5.3.6 Software Maintenance Mode (cont'd)

5.3.6.3 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:

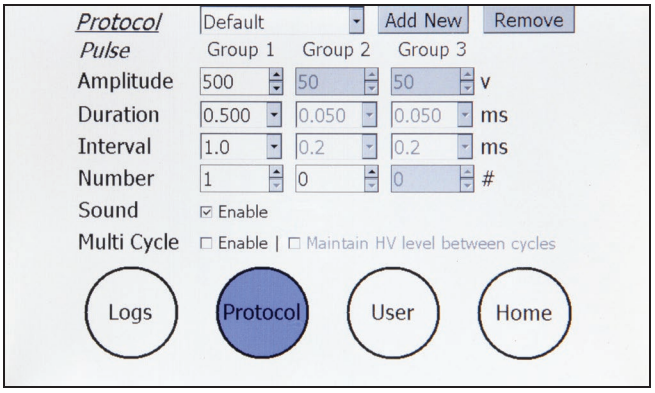


Figure 5-15: Pulse Protocol Modification

Protocol Parameter Boundaries					
	Amplitude (volts)	Duration (ms)	Interval (ms)	Pulse #	Pulse Groups
Minimum	50	0.050	0.200 (5 kHz)	1	1
Maximum	1000	10*	1000 (1 Hz)	10	3

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

*** 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.

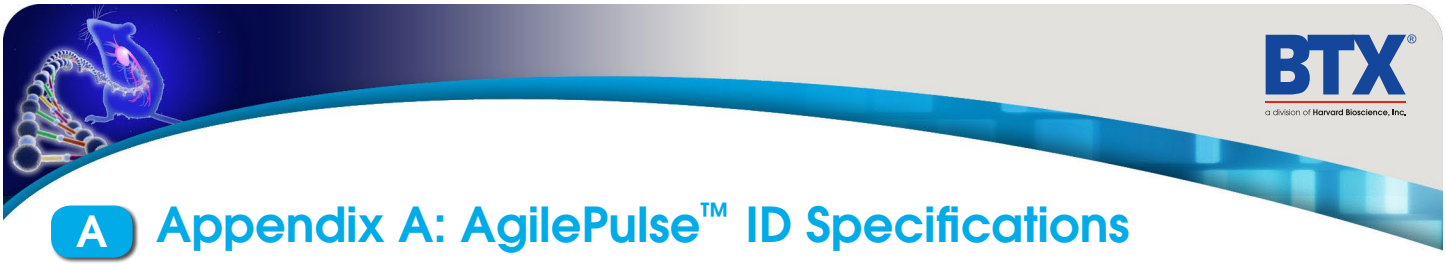
6 Customer Service

6.1 Limited Warranty

The terms of the warranty are covered in the BTX warranty.

6.2 Customer Service

If the user believes that there is a defect in the BTX product, the customer should contact BTX Technical Support at **800-272-2775** or email **techsupport.btx@harvardapparatus.com**. If the system needs to be returned to BTX, please contact Technical Support for a Return Material Authorization (RMA) number.



A Appendix A: AgilePulse™ ID Specifications

Operation

Delivery

Touch Screen

Opening Screen for parameter entry

Experiment entry
Transfectant entry
Electrode ID entry
Operator ID entry

Pulse Screen

LOAD – measure skin resistance every second and display
READY – Turn on high voltage power supply
START – Start pulsing
DONE – vaccination completed

Mode 2 – Setup by trained IT specialist

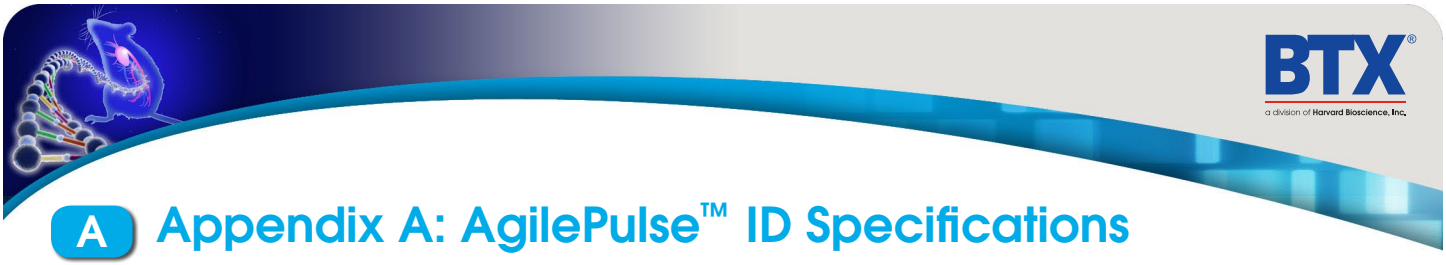
Pulse parameters

Download data files

Delivery Electrode

Vaccine Delivery Volume	2 blebs x 40 µl each	IDA-4-6
	2 blebs x 45 µl each	IDA-4-6
	2 blebs x 50 µl each	IDA-6-6
Delivery Target	Skin/dermis	
Electrode:		
Handle	Reusable with alcohol cleaning	
Tip	Sterile Single packaged Disposable	

	IDA-4-4-2	IDA-4-6-2	IDA-6-6-2
Row Spacing	4 mm	4 mm	6 mm
Needles/row	4	6	6
Needle Spacing	1.5 mm	1.5 mm	1.5 mm
Needle Diameter	0.3 mm	0.3 mm	0.3 mm
Needle Length	2 mm	2 mm	2 mm
V/d Maximum	2500 v/cm	2500 v/cm	1667 v/cm



A Appendix A: AgilePulse™ ID Specifications

AgilePulse Waveform Generator

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 µs to 10 ms	50 to 1000 volts 50 to 300 volts
Pulse Current Trip	26 amps	
Load Range	100 to 1500 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

Data Types

Raw Data	DV<Date>.xml
Log Data	DV<Date>.txt
CSV Data	DV<Date>.csv

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.

Front Panel

Computer:	
Operating System	Windows® Mobile 6.0
Interface	Touch screen
Line/Mains Switch with illumination	
Emergency Stop Button (resets computer to ready state)	
Touch Screen	
USB Ports	2
Electrode connector	Fischer Series 4032

Back Panel

Power Entry	IEC 320
Ethernet	RJ45 (NOT CURRENTLY INSTALLED)

Electrical and Mechanical

AgilePulse Cabinet with Handle (W x H x L)	16.5 cm x 30.5 cm x 40.6 cm 6.5 in x 12 in x 16 in
Weight	5.4 kg (12 lbs)
Operating temperature	10 to 40 oC
Mains Voltage	100 to 250 VAC
Fuse	5 Amp Slo-Blo®, 5 mm x 20 mm

Declaration of Conformity

Manufacturer: *Harvard Apparatus, Inc.*
84 October Hill Road
Holliston, Massachusetts 01746-1388, U.S.A.
Phone: (508) 893-8999

We herewith declare that the following product:

Product Name:	*BTX AgilePulse™ ID*	*BTX AgilePulse™ IM*	*BTX AgilePulse™ MAX*
Model No.:	*Catalog # 47-04xx*	*Catalog # 47-05xx*	*Catalog # 47-02xx*

To which this declaration relates, is in conformity with the applicable EC Directives, harmonized standards, and other normative requirements:

Application of Council Directive(s):	*2006/95/EC*	*Low Voltage Directive*
	2004/108/EC	*Electromagnetic Compatibility Directive*
	2011/65/EU	*RoHS Compliant*

Standard(s) to which conformity is declared:

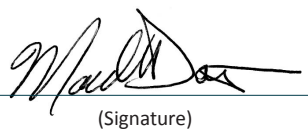
Safety:	*IEC 61010-1:2010 (3rd Edition)*
Emissions/Immunity:	*EN 61326-1:2013*
	FR47 FCC Part I5 Sub Part B (2011)
	EN 61000-4-2:1995 + A1:1998+A2:2001
	EN 61000-4-3:2002
	EN 61000-4-4:2004
	EN 61000-4-5:1995+A1:2001
	EN 61000-4-6:2007
	EN 61000-4-11:2004
	EN 61000-3-2:2000
	EN 61000-3-3:1995+A1:2001
	CISPR11:2003+A1:2004, Group 1, Class A

EMC and Safety compliance were evaluated by Intertek/ETL Semko

Reference test report file numbers: *100587731 BOX-001, -003, -004*

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directive(s) and Standard(s).

Place: *United States of America*
Date: *April 14, 2015*



(Signature)

Mark Davis

(Full Name)

Director, Global MPE

(Position)

CE

Harvard Apparatus