

## BTX-Division of Harvard Apparatus



**ECM<sup>®</sup> 2001 Plus**



**Hybrimmune<sup>™</sup>**

The logo for BTX Harvard Apparatus, featuring the letters 'BTX' in a large, bold, white font with a registered trademark symbol, and 'HARVARD APPARATUS' in a smaller, white, sans-serif font below it.

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The Electroporation Experts

CELL TRANSFECTION & CELL FUSION  
PRODUCTS

# Cell Fusion Theory

The formation of a single hybrid cell containing the nuclei and cytoplasm from different cells

- General Approach
  - Bring cells together so they touch
  - Compress the cells to increase surface area
  - Disrupt cell membrane
  - Provide a growth environment

# Cell Fusion Methods

**FUSION/Alignment Catalyst**

**Electricity/Electricity**

**Virus/Tissue Culture**

**PEG/Centrifuge**

## General Approach

1. Bring cells together so they touch
2. Disrupt cell membrane
3. Provide a growth environment

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## PEG vs. Electrofusion

PEG-Mediated Fusion	Electrofusion
Peroxide build up contributes to cell cytotoxicity.	Not cytotoxic; Physical method
Lower fusion efficiencies	Hybrid yields are up to 10-fold over PEG-mediated fusion <sup>8</sup>
>10 <sup>8</sup> cells are required for PEG-mediated fusion <sup>5</sup>	Fewer B cells required <10 <sup>7</sup>
Not reproducible: Too many variables (size & shape of the pellet; stirring method; technique varies from person to person) <sup>5,6,7</sup>	Optimized and reproducible protocols

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Hybrid yields generated by Electrofusion are  
10-fold greater than PEG

Experiment Number	Antigen Specific Clones	
	E-Fusion	PEG
1	20	0
2	10	0
3	400	23
4	151	21
Mean	145	11

Four different transgenic mice expressing Abs to human Ag were used to compare the efficiency of E-Fusion to PEG fusion.



## Electrofusion resulted in 9X more Ag<sup>+</sup> reactive hybridomas relative to PEG fusion

- 8-fold more clones were obtained using electrofusion vs. PEG (542 vs 58)
- 9-fold more hybrids tested positive to the specific antigen tested. Compared to those fused with PEG.(91 vs 12)

Experiment	E-fusion			PEG-fusion	
	Ag	#γ,k	#Ag,γ	#γ,k	#Ag,γ
1	TT	336	96	ND	ND
2	TT	170	40	ND	ND
3	TT	208	20	0	0
4	TT	1400	10	150	0
5	TT	<1100	<400	83	23
6	TT	582	151	69	21
7	Ag #1	456	85	8	1
8	Ag #2	ND	166	ND	18
9*	Ag #3	493	101	128	56
10	Ag #4	71	0	0	0
11	Ag #5	323	0	47	0
12	Ag #5	246	0	36	0

Average

542 91

58 12

Difference (fold)

9 8

\*: scoring criteria different 0.5 vs 0.3, and all Ag,γ were mouse

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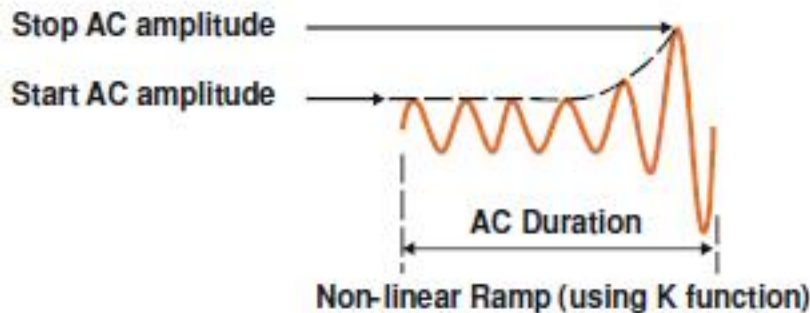
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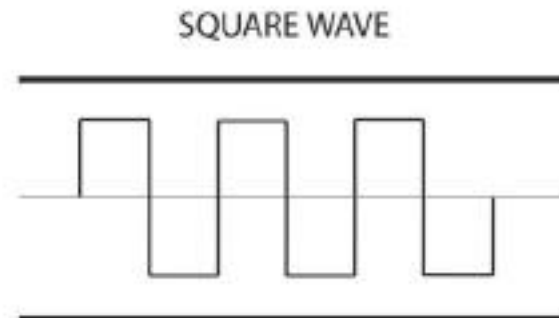
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# Electrofusion Mechanism

Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. The properties of two waveforms are used:



Oscillating AC Waveform



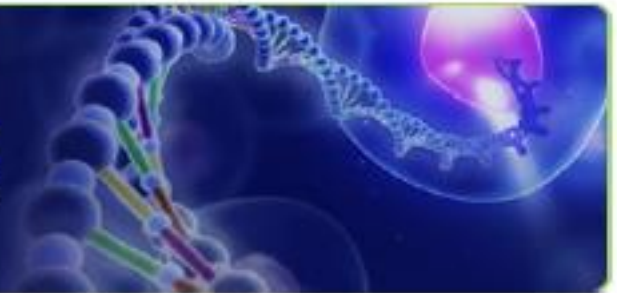
DC Square Waveform

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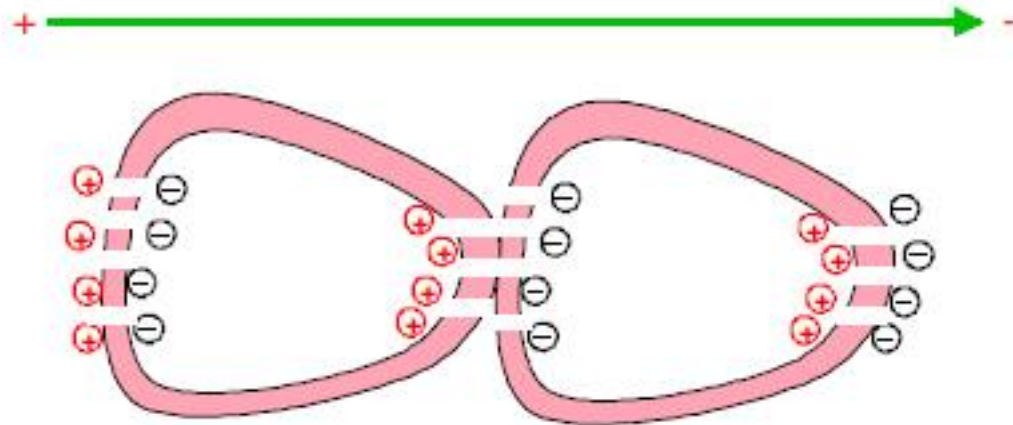
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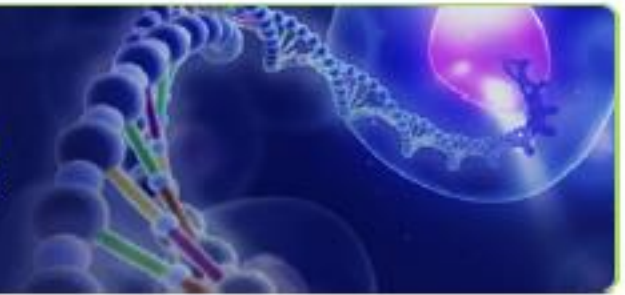


## Fusion Pulse Disrupt Membrane

Apply a brief but intense Electric Field to form temporary pathways in cell membrane

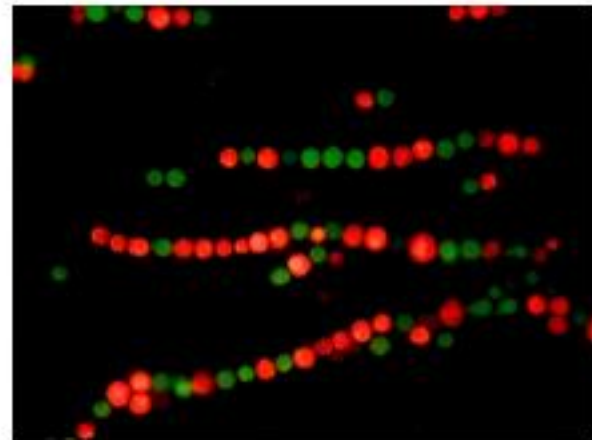
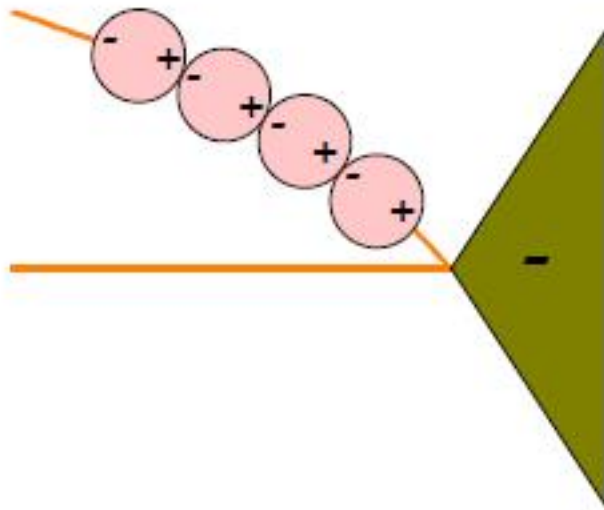






## Step 1-Pre-Pulse AC Cell Alignment

As cells move toward a common point  
the dipoles attract

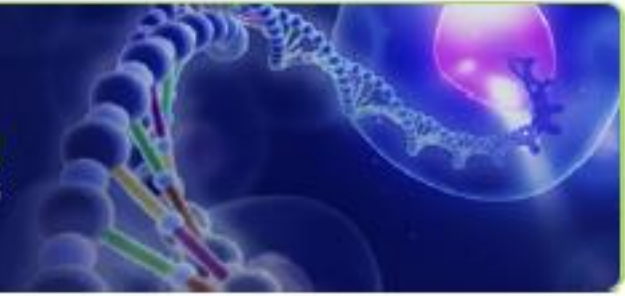


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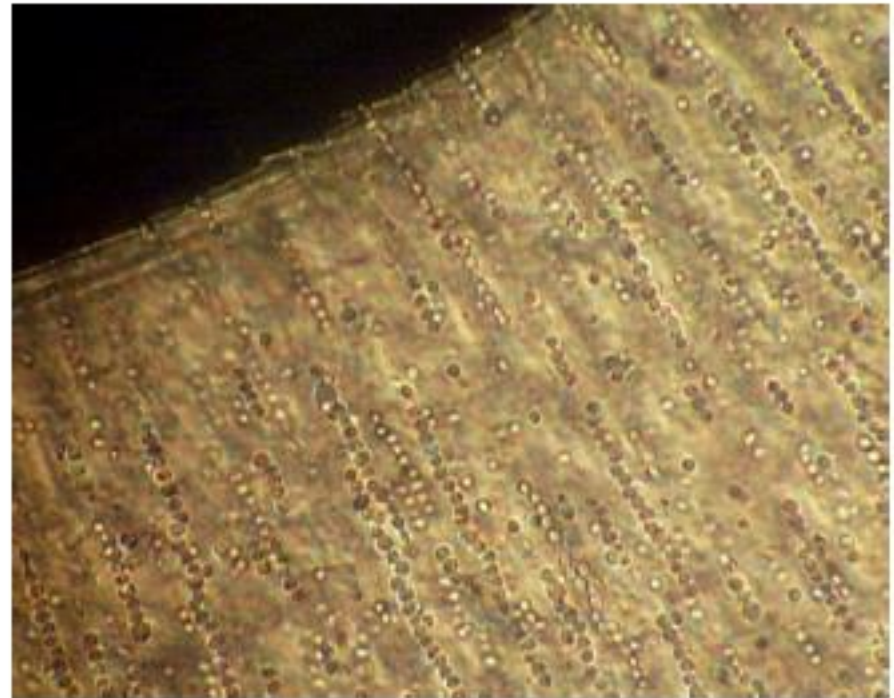
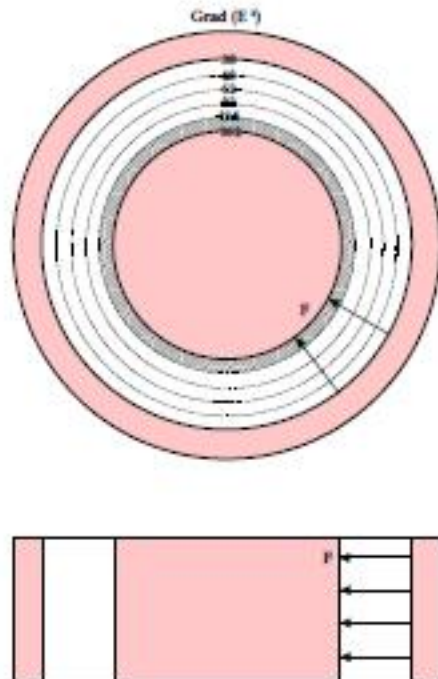
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## Coaxial Electrode ( $v^2/mm^3$ )



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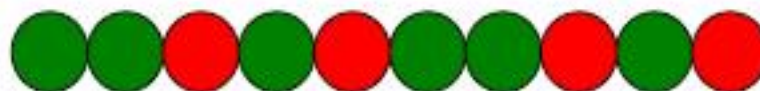
## Adjacent Cell Probability

$$XY = 25\%$$

$$YX = 25\%$$

$$XX = 25\%$$

$$YY = 25\%$$



$$XY + YX = 50\%$$

## Hybrimmune<sup>®</sup> Hybridoma Production System

- Tri-Phasic Waveform Generator
- Computer controlled (not included)
  - Windows Interface
  - Record Keeping: Saves protocols/logs
- Large volume chambers for production
  - 2ml chamber optimization
  - 9ml chamber for production
- BTXpress Cytofusion Media
  - Specially formulated
  - GMP Compliant

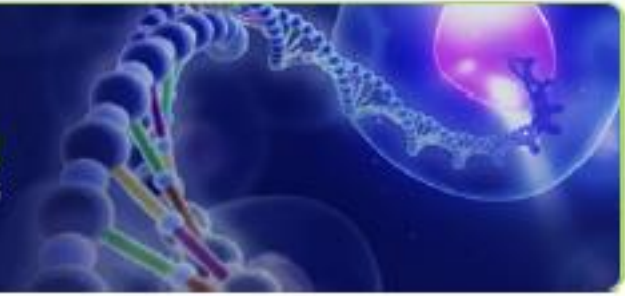


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## ECM 2001+ Enhanced Features

- Advanced protocol capabilities
  - Can combine up to 19 AC steps pre- and post- DC fusion step
- Enhanced AC sine waveform step programming capabilities
  - 0.2-2.0 MHz
  - 1-99 seconds
  - 5-75V, Linear ramping available
- New safety features, including pre-pulse sample resistance check, arc protection, and over current pulse abort.
- Large 7 inch touchscreen interface and integrated software within instrument
- Reduced footprint and weight
  - Length and width footprint space on bench matches current Gemini, however ECM 2001+ is taller in height.



# BTX<sup>®</sup> ECM 2001+ Software Preview

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## Main Menu





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## Electrofusion Main Menu

Main Electrofusion Menu

Quickstart

User Protocols

Preset Protocols

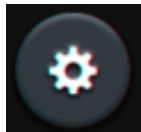
ECM 2001+  
Electrofusion

09/08/2012 3:01:38 PM

Settings (gear icon) Navigation (left arrow icon)

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## Device Settings Main Menu

The screenshot shows the 'Settings' menu for the ECM 2001+ Electrofusion device. The menu is displayed on a dark background with white and red text. On the left side, there are five large, dark buttons with white text: 'Update Software', 'Time/Date', 'Audible Alarms', 'Device Information', and 'Backlight: 80%'. On the right side, the device name 'ECM 2001+' is displayed in white, with 'Electrofusion' in red below it. A white box contains the text 'Date and Time not set.' Below this box is another empty white box. At the bottom right, there are two circular navigation buttons: a gear icon and a left-pointing arrow.

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## Quickstart Electrofusion

**AC**

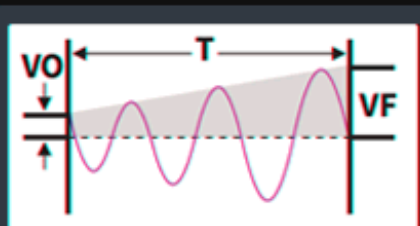
VO: 55 V

VF: 65 V

T: 20 s

F: 0.5 MHz

1/3



**DC**

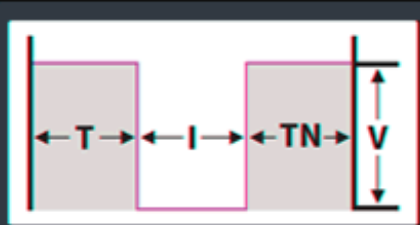
V: 505 V

T: 20  $\mu$ s

N: 2

I: 3.000 s

2/3



**AC**

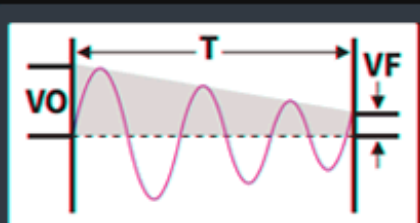
VO: 65 V

VF: 5 V

T: 5 s

F: 1.0 MHz

3/3

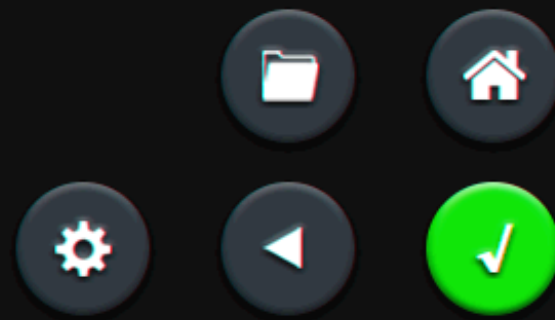


**ECM 2001+**

Electrofusion

Date and Time not set.

9 mL Coaxial Chamber



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## BETA CELL FUSION

Step 1/4 (Pre-AC):

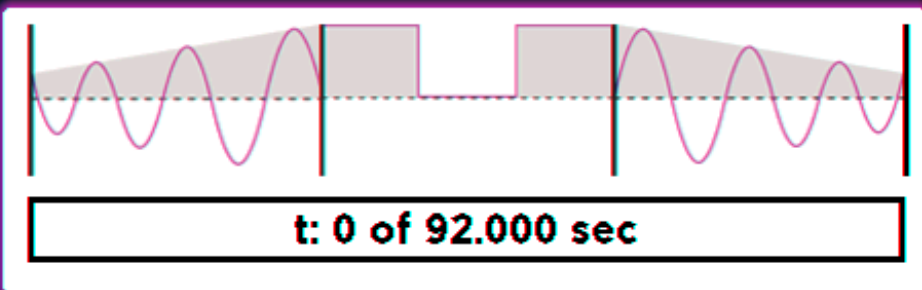
VO: 5 V; VF: 50 V; T: 30 s; F: 0.5 MHz

Step 2/4 (Pre-AC):

VO: 50 V; VF: 50 V; T: 30 s; F: 0.5 MHz

Step 3/4 (DC):

V: 450 V; T: 10  $\mu$ s; N: 3; I: 1.00 s



## ECM 2001+

### Electrofusion

09/12/2012

4:30:53 PM

Press Omega to measure load or the green "GO" button to start the protocol.

9 mL Coaxial Chamber

$\Omega$

Home icon

Settings icon

Back icon

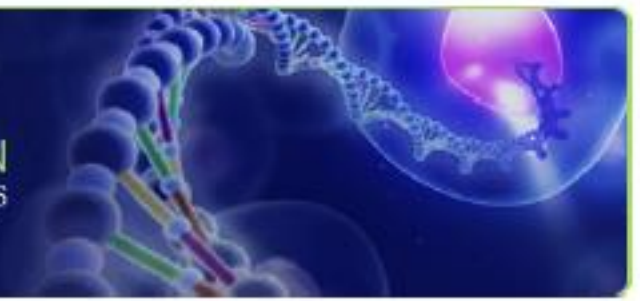
GO

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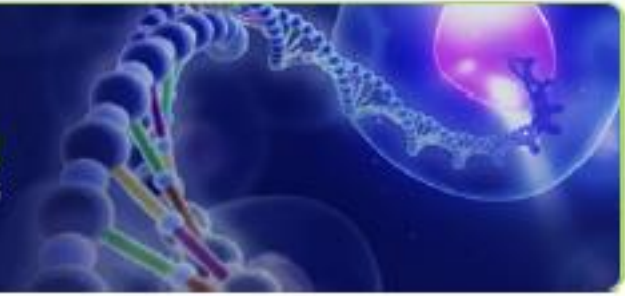


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# Positioning ECM 2001+ vs. ECM 2001 and Hybrimune

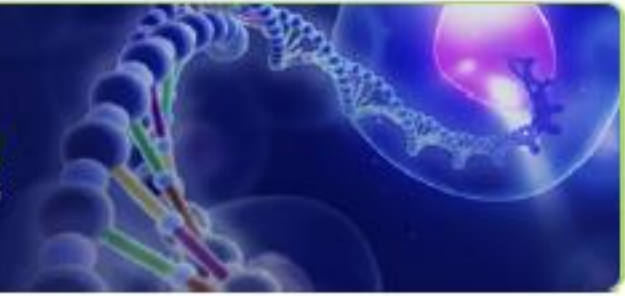
	ECM 2001+	Hybrimune	ECM 2001
<b>Target customers</b>	Biotech/Industry/Pharma, some Academic	Biotech/Industry/Pharma	Academic/Teaching
<b>Applications</b>	Broad Electrofusion and Electroporation, Hybridoma Creation	Hybridoma Creation	Broad Electrofusion and Electroporation
<b>Licensing Fee for Customer</b>	No	Yes, <b>15K USD annually paid to Collectis</b>	No
<b>User Interface</b>	Touchscreen	Connect to external PC (not included) running Hybrimune software	Manual buttons/knobs, Digital voltage displays
<b>AC waveform</b>	Sine wave, 0.2-2 MHz, 5-75 V peak, constant or linear ramping available, duration 0-99 s	Sine wave, 0.2-2 MHz, 5-75 V peak, constant, linear ramping, or exponential ramping available, duration 0-60 s	Proprietary waveform, 1.0 mHz fixed, 0-75 V <sub>rms</sub> (Equivalent to ~106 V peak), constant, pre-fusion duration 0-99 sec, post-fusion duration 0-9 s, post-fusion voltage 1/10 of pre-fusion amplitude.
<b>DC square waveform</b>	5-3000V, 10 μs-999 ms	100-1000 V, duration 20-1000 ms	10-3000V, 10 μs-999 ms
<b>Advanced programming</b>	Yes; 1 DC step with up to 19 different AC steps total that may occur in any combination pre- and/or post- DC	Yes; Up to 10 different groups total, each group containing 0-1 each of 3 parts ordered AC/DC/AC	No; AC/DC/AC program with option of up to 9 additional sequential repeats of same fixed program
<b>User protocols, Log file storage</b>	Yes, >1000 on instrument SD card	Yes, stored on customer's PC	No
<b>Preset protocols</b>	Yes, >20	No	No

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***Thank you***