Document number: 40203-01-EN
Version: 1
Valid from: SW 2.1

REFERENCE HANDBOOK

BactoSense Multi

Rapid bacterial monitoring system

bNovate Technologies SA
Ch. Dent d'Oche 1A
CH-1024 Ecublens
Switzerland

Tel.+41 (0)21 552 14 21
info@bnovate.com
www.bnovate.com

Table of Contents

1 General user information

1.1 Purpose of the reference manual

This reference manual provides the user with more detailed information that supplements the instruction manual.

The reference manual is intended for all persons who are familiar with the contents of the instruction manual and require detailed information about subjects such as design, configuration and repairs. The intented use of the BactoSense Multi is described in 40202 Instruction Manual BSM.

This document is part of the product. It should be stored in a safe place and always be close at hand for the user. The most recent version of this document can ordered from a bNovate Technologies representative in your country www.bnovate.com/distribution-partners.

40202	Instruction Manual BSM	Information on the life cycle of the BactoSense Multi. Intended use of the device.
40201	Quick Start Guide BSM	Basic information needed to quickly operate the BactoSense Multi.
40205	Cleaning Kit BSM	Usage of the Cleaning Kit for the BactoSense Multi
30201	Data Sheet BSM	Descriptions and technical data about the BactoSense Multi.
41221	Declaration of Conformity	Compliance with the underlying directives and standards.
41421	CB Test report	UL/CSA/FCC compliance report, also under CH-11152 on https://certificates.iecee.org

1.2 Safety symbols

All **safety symbols** used in this document are explained below:

DANGER!

Electric shock that may result in serious injury or death.

Ignoring this notice may lead to electrical shocks and death.

EXPLOSION HAZARD!

Explosion that may result in serious injury or death.

Ignoring this notice may cause explosions resulting in serious property damage and death.

WARNING!

Injury or hazards to health with long-term effects.

Ignoring this warning may lead to injuries with possible long-term effects.

CAUTION!

Material damage.

Ignoring this notice may cause material damage to the instrument and its peripherals.

1.3 Pictograms

All **pictograms** used in this document are explained below:

Additional information about the current topic.

Practical procedures when working with the BactoSense.

The screenshot is an example and may differ from current device.

2 Measurements

2.1 Measurement result

This page shows the result of the latest measurement. From here, the user can delete a measurement, look up older results (and export them), or export this result. Expert users can repeat the cell counting with new gates. Some actions are bound to specific accounts (Basic, Advanced or Admin).

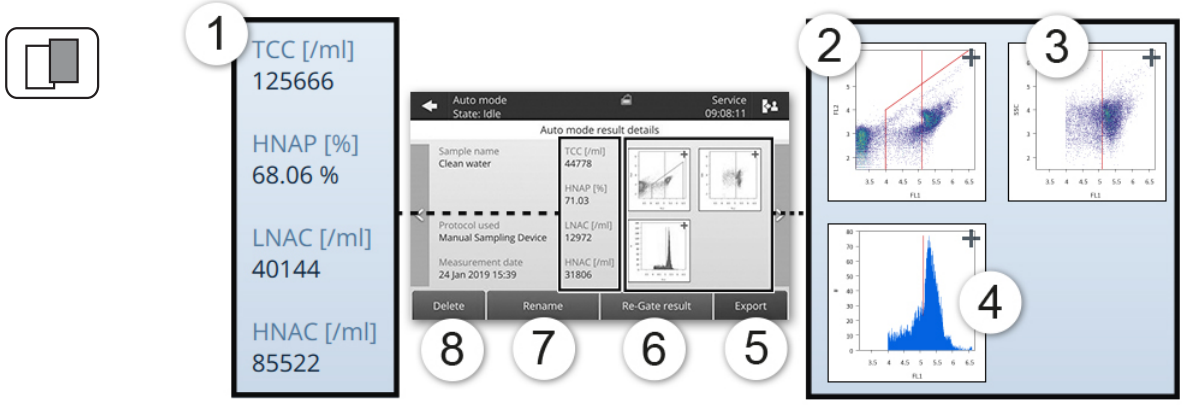


Figure 2 - 1 : Display of a single measurement result, using a TCC cartridge

①	Measured parameters are displayed. For details refer Section 2.2.
②	The FL2 vs FL1 dotplot shows all detected events according to the amplitude of their fluorescence signals FL1 (535 nm, X-axis) and FL2 (715 nm, Y-axis). The red polygon defines the gate. For details refer Section 2.4 and Section 2.5.
③	The SSC vs FL1 dotplot shows only cells inside the gates, according to their fluorescence signal FL1 (535 nm) and scattered light signal SSC (488 nm).
④	The FL1 histogram shows all cells inside the gates, binned according to their fluorescence in FL1.
⑤	**Export** saves this result to a USB stick. To export multiple results, refer Section 2.6.1 and Section 2.6.2.
⑥	**Re-Gate result** allows you to move the gates and recalculate cell counts. You can optionally save the new gates for future measurements. For details refer Section 2.4.
⑦	**Rename:** ability to rename the result.
⑧	**Delete:** the result is deleted permanently (requires confirmation).

Multiple selection & batch operations: long-press to select multiple measurements, then **Delete / Re-gate / Export** selection.

2.2 Measurement parameters

2.2.1 TCC cartridge

With a TCC cartridge, the following parameters are displayed:

PARAMETER	UNIT	NAME	DESCRIPTION
TCC	1/ml	Total Cell Count	Total number of bacteria detected inside the TCC gate. It is an addition of HNAC and LNAC: $$TCC = HNAC + LNAC$$
HNAP	%	High Nucleic Acid Percentage	The percentage of HNA cells relative to TCC: $$HNAP = \frac{HNAC}{TCC} \cdot 100$$
LNAC	1/ml	Low Nucleic Acid Count	The number of cells inside the TCC gate, but below the HNA/LNA boundary.
HNAC	1/ml	High Nucleic Acid Count	The number of cells inside the TCC gate and above the HNA/LNA boundary.

For further information on how to adjust the gates see Section 2.3.

2.2.2 ICC cartridge

With an ICC cartridge, the following parameters are displayed:

PARAMETER	UNIT	NAME	DESCRIPTION
ICC	1/ml	Intact Cell Count	Total number of intact or living bacteria inside the ICC gate: $$ICC = HNAC + LNAC$$
HNAP	%	High Nucleic Acid Percentage	The percentage of HNA cells relative to ICC: $$HNAP = \frac{HNAC}{ICC} \cdot 100$$
LNAC	1/ml	Low Nucleic Acid Count	The number of LNA cells inside the ICC gate, but below the HNA/LNA boundary.
HNAC	1/ml	High Nucleic Acid Count	The number of HNA cells inside the ICC gate and above the HNA/LNA boundary.

For further information on how to adjust the gates see Section 2.3.

2.3 Adjust gate settings

Following flow cytometry standards, the BactoSense uses gates to count cells in samples. We define:

- **Gate**: a line or polygon delimited on the dotplots.
- **Set of Gates**: a collection of two or more gates, used for the gating strategy. The types of gates are fixed, but users can change the limits of each gate. These are different for TCC and ICC measurements.
- **Gating Strategy**: defines how the gates are combined to count cells. For example, HNAC is the number of cells that are inside the TCC polygon and higher than the HNA limit. These strategies are different for TCC and ICC measurements.

Users can copy and modify the default gates, for example to use different gates for each type of water. The gates are modified in the **Gate settings** menu. When launching a protocol or re-gating a measurement, the instrument will allow you to choose one of the gate sets that is compatible with the current cartridge.

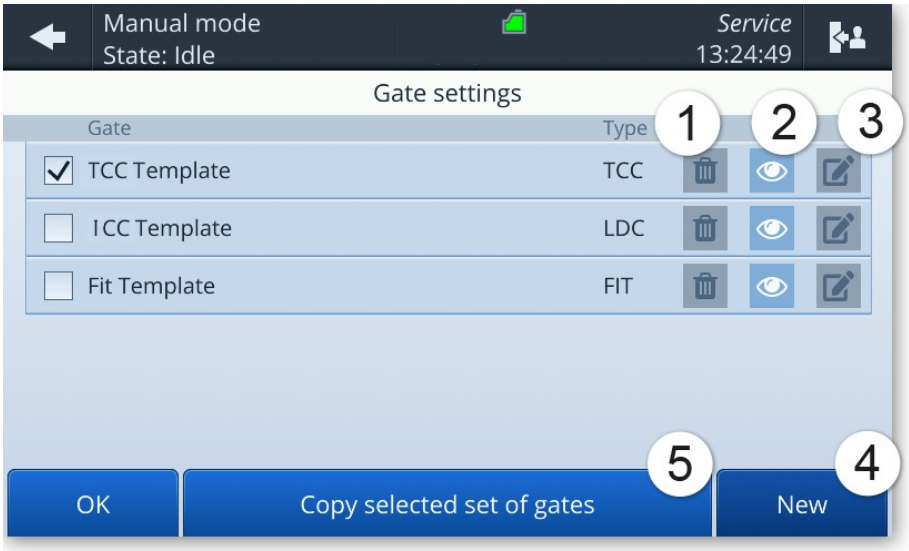


Figure 2 - 2 List of gate sets

①	Delete a set of gates. Templates cannot be deleted.	②	Preview the set of gates.
③	Modify the set of gates: change name or gate boundaries. The type (TCC, ICC) cannot be changed - for this, create a new set.	④	Create a new set of gates from scratch.
⑤	Copy an existing set of gates.		

The BactoSense knows four types of gate sets:

- **TCC**, used with the TCC cartridge.
- **ICC**, used with the ICC cartridge.
- **FIT**, used only for beads validation protocols, with any cartridge.

TCC gate sets contain two gates:

- **TCC**: a polygon defined on the FL1-FL2 plane. Points inside this polygon are counted as cells (TCC)
- **HNA** limit: a threshold on FL1. Points within TCC but larger than the HNA limit in FL1 are counted as HNAC. Points within TCC but smaller than the limit are counted as LNAC.

ICC gate sets contain two gates:

- **ICC**: a polygon on FL1 and FL2. Points inside the polygon are counted as Intact Cells (ICC)
- **HNA** limit: a threshold on FL1. Point within ICC but larger than the limit in FL1 is counted as HNAC. Points within ICC but smaller than the limit in FL1 are counted as LNAC.

2.3.1 TCC / ICC Gates

BactoSense, when using a TCC (respectively ICC) cartridge, defines two gates to count cells. The first gate, TCC for Total Cell Count (respectively ICC for Intact Cell Count), is a polygon defined on the FL1 vs FL2 dotplot. All points that fall within this polygon are counted as cells; all points that fall outside of it are ignored. The polygon can be defined by three to six points.

The second gate, the HNA limit, separates cells within TCC (respectively ICC) into two groups: Low Nucleic Acid cells (LNA) and HNA (High Nucleic Acid) cells. All data dots contained within the TCC gate, but with FL1 amplitudes larger than the HNA limit are counted as HNA cells, and all dots within the TCC gate with FL1 amplitudes smaller than the HNA limit are counted as LNA cells.

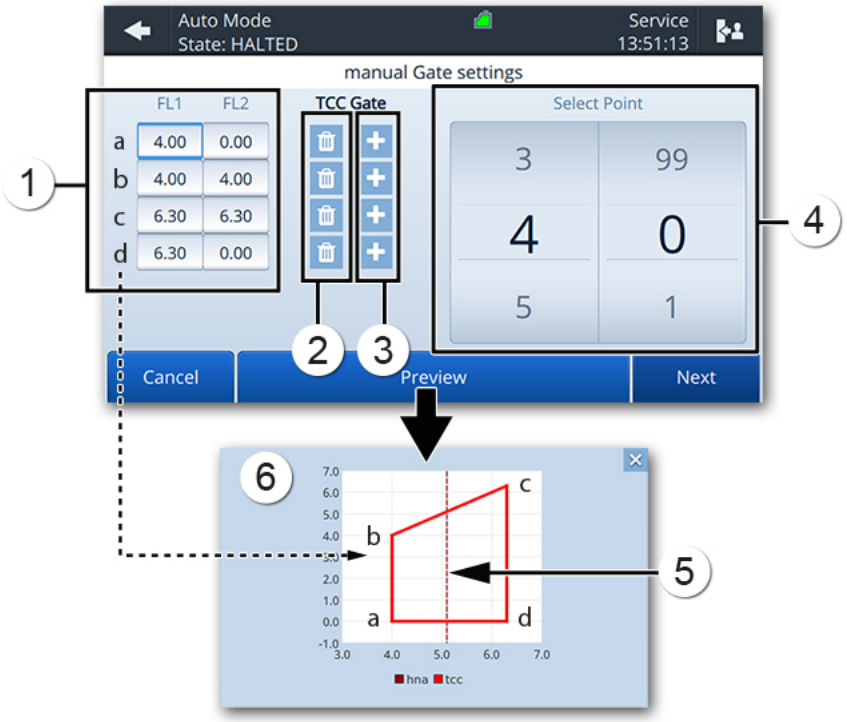
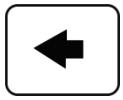
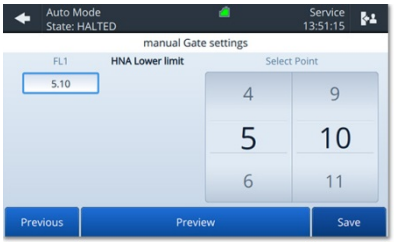


Figure 2 - 3 Gate settings for TCC

①	Coordinates of the gate's points. a: First point in the gate b: Second point in the gate c: Third point in the gate d: Fourth point in the gate	②	Remove a point from the gate.
③	Add a new point to the gate.	④	Modify the selected coordinate. In this screenshot, the FL1 (a, FL1) coordinate of the first point is selected, so we can move the first point in the gate along the FL1axis (x-axis).
⑤	HNA/LNA boundary: this gate is defined on the next screen.	⑥	Preview.

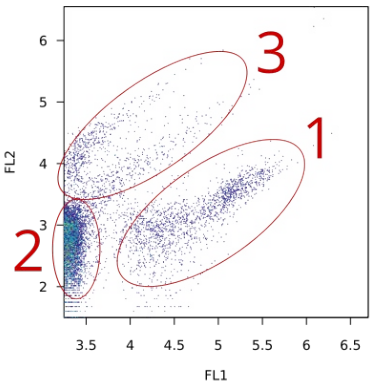
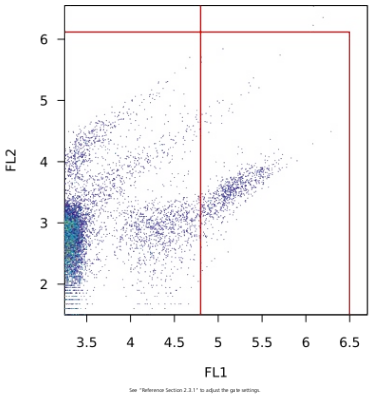
The following procedure describes how to adjust the gates:

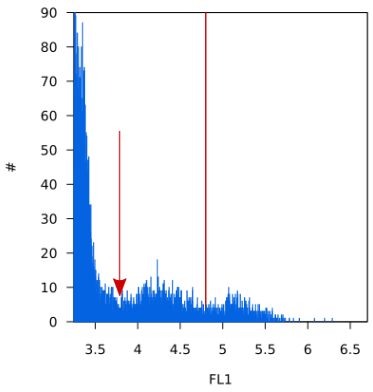
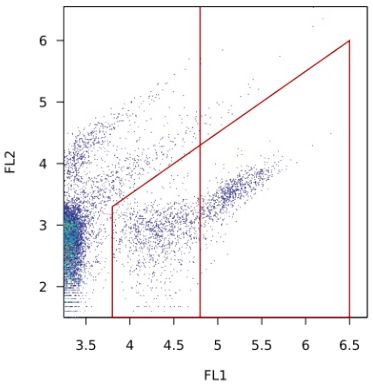
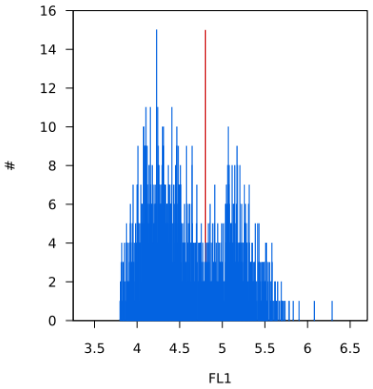
	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **Gate settings** button.	
3.	Press the **Edit** button of the gate you wish to edit.	
4.	Change the name if desired, then press the **Next** button.	
5.	Adjust the TCC/ICC gate limits according to Section 2.4.1.	
6.	Press the **Next** button.	
7.	Enter the HNA lower limit.	
8.	Press the **Save** button.	

2.4 Define the gate

2.4.1 Using TCC / ICC cartridges

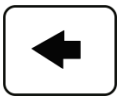
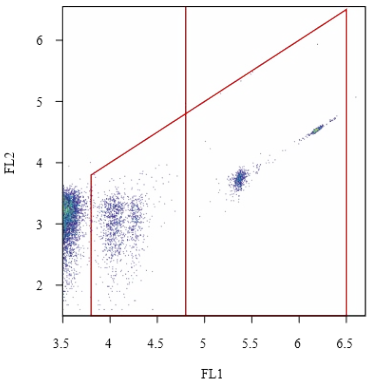
The BactoSense is delivered with default gate sets that are adequate for most of the situations. If needed, advanced users have the possibility to adjust the gate at any time once the measurement is done.
The procedure to adjust the gate is explained below and is valid for both TCC and ICC cartridges.

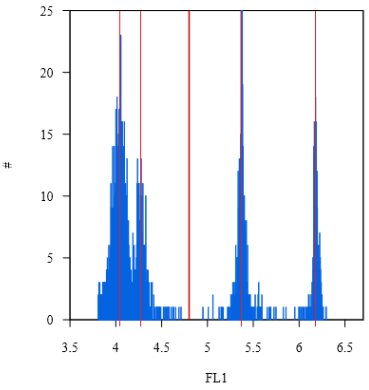
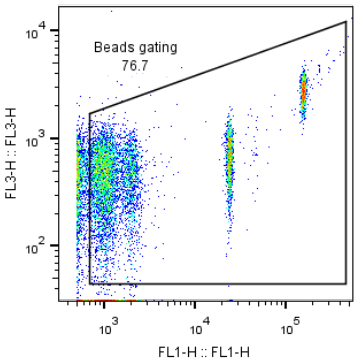
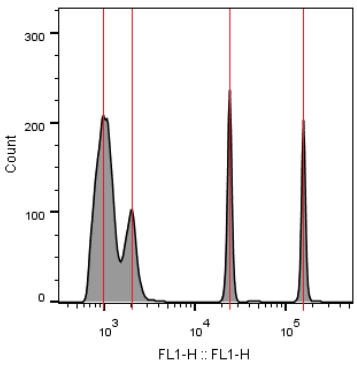
<table>
<tr><th></th><th>WORK STEP</th><th>ADDITIONAL INFO / IMAGE</th></tr>
<tr><td>1.</td><td>Prepare your sample and start a Water Analysis protocol.
On the Result details page, the FL1 vs FL2 dotplot shows:
1) Bacteria LNA and HNA in the water
2) Electrical noise from the optical detectors
3) Debris/background noise (These may include damaged cells, stating aggregates or particles)</td><td> 
</td></tr>
<tr><td>2.</td><td>Open the Gate settings menu and copy the desired gate template (Section 2.3). Give it a memorable name.</td><td></td></tr>
<tr><td>3.</td><td>Press on the icon “modify the set of gates” to adjust the gate (Section 2.3).</td><td></td></tr>
<tr><td>4.</td><td>Give a name to this set of gates and press Next.</td><td></td></tr>
<tr><td>5.</td><td>Place the gate at each extremity (left and right). For example, use these four points:
<table><tr><th>FL1</th><th>FL2</th></tr><tr><td>3.0</td><td>0.0</td></tr><tr><td>3.0</td><td>6.1</td></tr><tr><td>6.5</td><td>6.1</td></tr><tr><td>6.5</td><td>0.0</td></tr></table></td><td> 
</td></tr>
<tr><td>6.</td><td>On the Result details of your last measurement, Press the Re-Gate button.</td><td></td></tr>
<tr><td>7.</td><td>Select the gate you just created, and re-process the data with the new gate by pressing the button Confirm.</td><td></td></tr>
</table>

	WORK STEP	ADDITIONAL INFO / IMAGE
8.	Locate the separation between noise and data: open the histogram and locate the first trough after the noise peak, i.e., the first minimum after the peak at the extreme left (arrow in the picture). Write down the FL1 value of this minimum.	
9.	Return to **Gate settings** and edit your new gate. Place the left limits of the gate at the previously determined FL1 value. This will exclude the electrical noise of the optical detectors. If needed, for the FL2 axis, place the points "b" and "c" of the gate just below the debris/background area (generally appearing as straight diagonals). Save the gate, and re-gate your latest measurement once more.	
10.	To place the HNA/LNA limit, open the new histogram and locate the minimum between the LNA and HNA peaks (vertical line on the image here). Write down its FL1 value. In **Gate settings**, edit your latest gate once more, and use this FL1 value as the HNA/LNA limit. Re-Gate your measurement one final time.	

2.5 Comparison of measurement scales

To compare results of the BactoSense to another flow cytometer, a reference solution containing auto fluorescent beads needs to be analyzed. This method allows the user to compare the measurement scales of different devices.

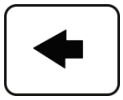
	WORK STEP	ADDITIONAL INFO / IMAGE
1.	It is recommended to use the Validation Kit which contains ready to use beads solutions.	Read the instructions of the **Validation Kit** for more information.
2.	Install the manual sampling device according to the Instruction Manual.	
3.	Take of one of the Validation kit's beads solutions and load it into the sampling device.	Read the notice of the **Validation Kit** for more information.
4.	Press the **Home** button. Press the **Back** button as many times as needed for the **Home** button to appear.	 
5.	Select **Manual mode** and confirm with **Go**.	Instruction Manual
6.	Choose the **Prime** protocol from the list.	The available protocols are described in the Instruction Manual.
7.	Press the **Next** and **Start** button. Let the priming protocol finish.	
8.	Choose the **Beads Analysis** protocol from the list and run it.	
9.	Wait for the results and if necessary, precisely adjust the gate of the FL1 vs FL2 dot plot to count only the 4 populations of beads. It is important to ensure that noise, background, and debris are not counted into the gate.	See Section 2.3.1 to adjust the gate. 

	WORK STEP	ADDITIONAL INFO / IMAGE
10.	On the FL1 vs counts histogram, estimate the log value of each peak's center on the FL1 axis. In this example, we obtain approximately (in log): 1st peak = 4.05 2nd peak = 4.3 3rd peak = 5.35 4th peak = 6.2	
11.	Measure the same beads with the other flow cytometer you want to compare and repeat the steps 10 and 11. In this example, we obtain approximately (in log): 1st peak = log(10^3) = 3 2nd peak = log(2*10^3) = 3.3 3rd peak = log(2.4*10^4) = 4.4 4th peak = log(1.6*10^5) = 5.2	 
12.	With the different values obtained for both devices, it is now possible to compare their scales and their measurement windows.	

2.6 Export data

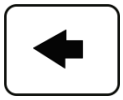
2.6.1 Export single measurement

The export function saves data to an USB flash drive or hard drive. It creates a folder for the selected measurement, which contain its FCS file and plots in PNG format. Additionally, CSV and Excel files are generated, containing a summary of this measurement result (TCC, ICC, HNAC, LNAC, HNAP).

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home button** to get to the Home menu, select **Manual mode** and press **Go** (Instruction Manual). To export results from Auto mode, select **Auto mode** instead. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **View Results** button.	
3.	Find your result and press on it, leading to the result overview.	
4.	Press the **Export** button.	
5.	Connect the USB mass storage device (Instruction Manual). If needed, press the **Refresh USB list** button until the stick is detected. ⚠ USB flash drive should be formatted in FAT32 which is the common standard.	
6.	Enter a folder name and press **Export Results (dot plots) or Export all (dot plots, FCS and debug files)**	If the available storage capacity of the USB mass storage device is too small "disk out of space" is displayed.
7.	The data are copied to the USB mass storage device. ⚠ Do not remove the USB mass storage device during the data transfer.	

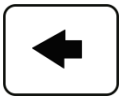
2.6.2 Export measurement series

The export function saves data to a USB flash drive or hard drive. It creates one folder per measurement, which contain the FCS file and plots in PNG format. Additionally, CSV and Excel files are generated, containing a chronological list of all measurement results (TCC, HNAC, LNAC, HNAP, ICC).

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	To export data obtained in the Manual mode press the **Home** button to get to the Home menu, select **Manual mode** and press **Go** (Instruction Manual). To export results from Auto mode, select **Auto mode** instead. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **View Results** button.	
3.	Press the **Export Series** button.	
4.	Optionally, choose the date range of measurements to be exported and press **OK**.	
5.	Insert the USB mass storage device (Instruction Manual). If needed, press the **Refresh USB list** button until the stick is detected. ⚠ USB flash drive should be formatted in FAT32 which is the common standard.	
6.	Enter a folder name and press **Export Results (dot plots) or Export all (dot plots, FCS and debug files)**	Each measurement will be saved as a sub-folder within this folder. If the available storage capacity of the USB mass storage device is too small “disk out of space” is displayed.
7.	⚠ Do not remove the USB mass storage device during the data transfer. The data are copied to the USB mass storage device.	

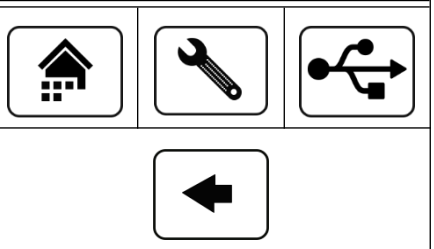
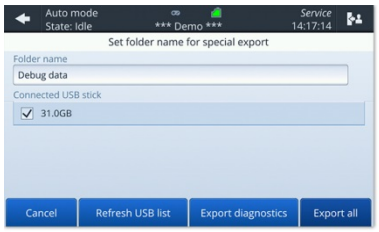
2.6.3 Export selected measurements

The export function saves data to an USB flash drive or hard drive. It creates a folder for the selected measurement, which contain its FCS file and plots in PNG format. Additionally, CSV and Excel files are generated, containing a summary of this measurement result (TCC, ICC, HNAC, LNAC, HNAP).

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home button** to get to the Home menu, select **Manual mode** and press **Go** (Instruction Manual). To export results from Auto mode, select **Auto mode** instead. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **View Results** button.	
3.	Press and hold the first result you want to export. When the check box appears, you can select other results. The Action bar shows actions that can be applied to multiple results at once: re-gate, export, and delete.	
4.	Press the **Export** button.	
5.	Connect the USB mass storage device (Instruction Manual). If needed, press the **Refresh USB list** button until the stick is detected. ⚠ USB flash drive should be formatted in FAT32 which is the common standard.	
6.	Enter a folder name and press **Export Results (dot plots) or Export all (dot plots, FCS and debug files)**	If the available storage capacity of the USB mass storage device is too small "disk out of space" is displayed.
7.	The data are copied to the USB mass storage device. ⚠ Do not remove the USB mass storage device during the data transfer.	

2.6.4 Export all data or diagnostic data

The "Special import/export" function allows users to export either all measurement data from the instrument, or to export only diagnostics data which can be interpreted by service technicians.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button to get to the **Home** menu, select **Maintenance** and **Special export import** button ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	
2.	Connect the USB mass storage device (Instruction Manual). If needed, press the **Refresh USB list** button until the stick is detected. ⚠ USB flash drive should be formatted in FAT32 which is the common standard.	
3.	Enter a folder name.	
4.	To export all data, press the **Export all** button. To export only diagnostics data, press the **Export diagnostics** button. ⚠ Do not remove the USB mass storage device during the data transfer.	Exporting all data can take several hours, since there can be up to 30 GB of data in tens of thousands of small files. If the available storage capacity of the USB mass storage device is too small "disk out of space" is displayed.

2.6.5 Manage services with FTP

Data can be remotely accessed using the FTP protocol (RFC 3659). Authenticate with the same users and passwords as the authentication on the instrument itself or on the web user interface.

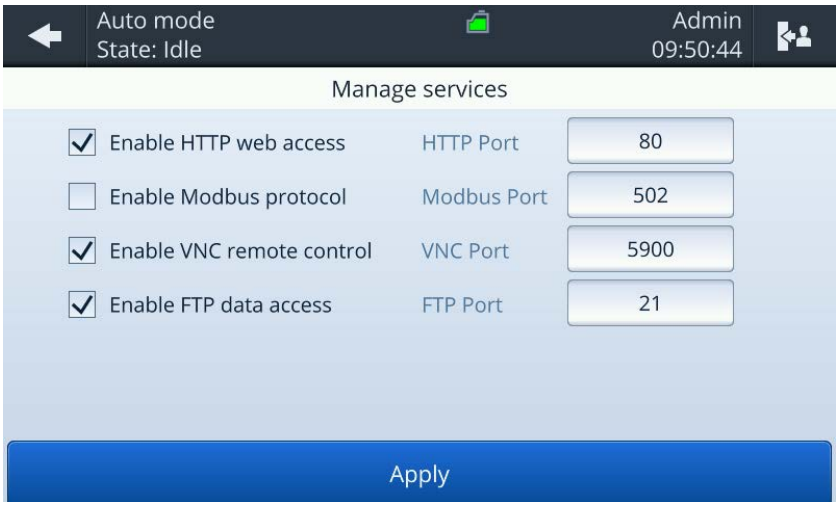
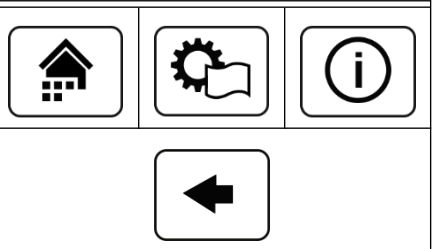


Figure 2 - 4 Manage services

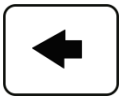
The CSV and Excel files are generated on-the-fly, and thus always contain the latest measurement data.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button to get to the **Home** menu, select **System settings** and **Services** ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	
2.	Check the box **Enable FTP data access**	
3.	Chose a port for the FTP server	ⓘ The default port is port: 21
4.	Press the **Apply** button to reboot the service	
5.	Access the data using a FTP client or Windows using the following scheme: ftp://user:password@ip:port	

3 Settings

3.1 Demo mode

Demo mode can be used when demonstrating the device or when learning how to use the menus. When it is activated, the instrument reboots into a special mode that displays demonstration data (the owner's measurement results are hidden). All the instrument's functions are replaced by simulations that never move the hardware or modify the results database. This means protocols can be started without needing to load a sample, and users can pretend to delete or re-gate results without ever changing the data. If the demo mode is activated, the message ***Demo*** is displayed in the upper middle of the screen.
When the instrument is reverted to normal mode, the owner's data is shown again, and all functions are active again.

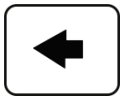
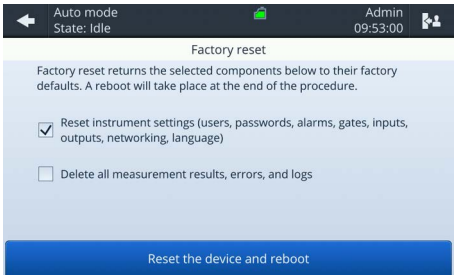
	WORK STEP	ADDITIONAL INFO / IMAGE	
1.	Press the **Home** button. Press the **Back** button as many times as needed for the **Home** button to appear.		
2.	Enter the **System settings** then **Demo mode** menu.		
3.	Select the **Activate demo mode** box.		
4.	Press the **Save and restart** button.		
5.	The device reboots automatically.		

To return into normal mode, follow the same procedure, but uncheck the **Activate demo mode** box.

3.2 Factory reset

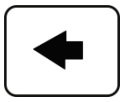
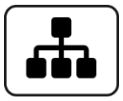
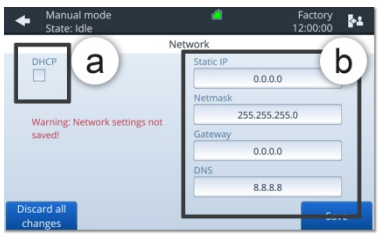
Factory reset reverts most of the instrument settings to the factory values: gating limits, measurement interval, default protocol names, users, language and network settings. This option is accessible only by the Admin and Service logins.

The following procedure describes how to perform a factory reset:

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Enter the **System settings** then **Factory reset** menu.	 
3.	Select which parameters you wish to reset: either the instrument's settings, either all measurements, either both of them.	
4.	The device reboots automatically.	

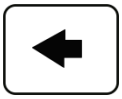
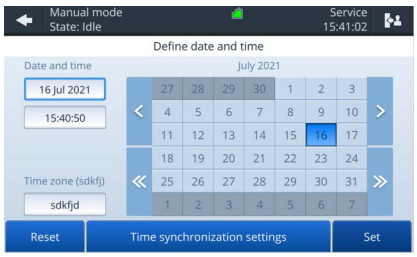
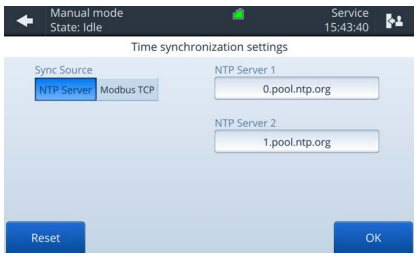
3.3 Network configuration

Ask your network administrator for the correct settings.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **System settings** button.	
3.	Press the **Network** button.	
4.	A: For **Dynamic IP** check the **DHCP** check box (a). B: For **Static IP** uncheck the **DHCP** check box (a) and enter the network details (b).	
5.	Press the **Save** button.	
6.	Reboot the device.	

3.4 Set NTP Servers

NTP is a computer network protocol which is used to synchronise time on computers across a network. By default, these are set to synchronise to the Network Time Foundation servers (ntp.org). You have the possibility to set custom NTP servers in the Time menu.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **System settings** button.	
3.	Press the **Date & Time** button.	
4.	Press **Time synchronisation settings** buttton	
5.	Select the desired sync Source	NTP
6.	Update NTP servers	
7	Press the **OK** button.	ⓘ By editing and changing a server address and pressing **Ok**, will automatically ping the server and update the time

3.5 Service information

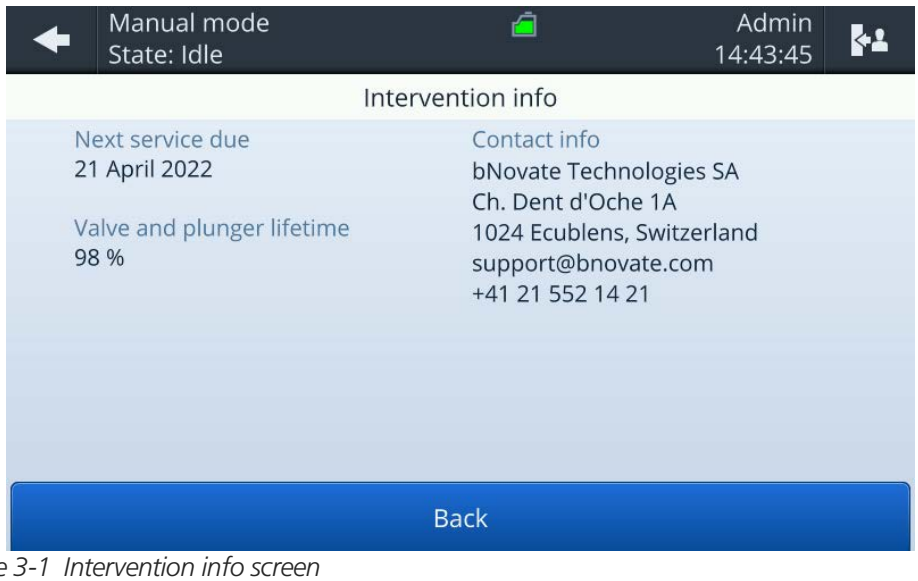
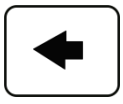
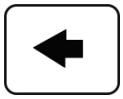


Figure 3-1 Intervention info screen

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **Maintenance** button.	
3.	Press the **Intervention info** button.	
4.	An intervention should be scheduled before the next service due date or before the valve and plunger lifetime reaches 0%. If a service is needed a warning will be attached to the measurement result.	

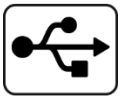
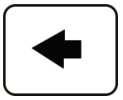
3.6 Service interventions log

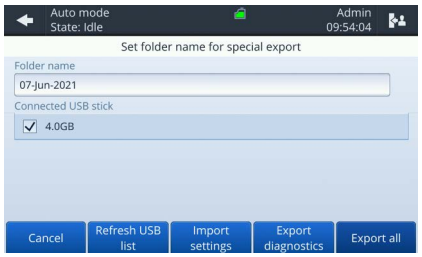
The following procedure describes how to view details of past service interventions.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button. ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	 
2.	Press the **Maintenance** button.	
3.	Press the **Intervention log** button.	
4.	Past service interventions are listed in this log screen.	

3.7 Import / Export settings

Settings are exported to a USB drive as per section 2.6.4. The settings are compiled to a timestamped file ending with ***.bnv***. In order to import the settings in a new instrument, or as a backup, it is necessary to copy the ***.bnv** file at the root of the USB drive, or in the directory the file was exported to. If multiple copies of the settings are located in one USB drive, the system will use the most recent one.

	WORK STEP	ADDITIONAL INFO / IMAGE
1.	Press the **Home** button to get to the **Home** menu, select **Maintenance** and **Special export import** button ⓘ Press the **Back** button as many times as needed for the **Home** button to appear.	   
2.	Connect the USB mass storage device (Instruction Manual). If needed, press the **Refresh USB list** button until the stick is detected. ⚠ USB flash drive should be formatted in FAT32 which is the common standard.	Make sure the ***.bnv** file is at the root of the USB drive

	WORK STEP	ADDITIONAL INFO / IMAGE
3.	Press **Import settings**	
4.	Select the following settings you want to import. Measurement settings include gate sets, IO settings, alarms, and date and time settings. Network settings comprise IP, DNS, and NTP addresses	
5.	Press **Import Settings** and wait until the device reboots	

4 Web user interface

4.1 General information

- The web interface allows users to browse and export results from a remote computer, as well as monitor the state of the instrument (cartridge level, errors, etc.)
- The BactoSense needs to be on the same network as the computer that accesses the web interface.
- The web interface can be loaded on any browser on Desktop computers or Mobile devices.
- Take care about data and network security with all usual measures (Instruction Manual).

4.2 Connect to the web interface

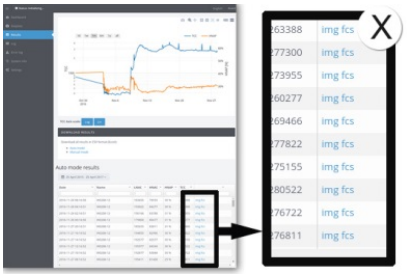
	WORK STEP	ADDITIONAL INFO / IMAGES	
1.	Find the IP address of the BactoSense. Press the **Home** button. Press the **System info** button. The IP address is on the system info page.		
2.	On your computer, open a web browser.		
3.	Enter the IP address in the address bar and press enter.		
4.	Enter your login; the main page should appear.		

4.3 Start page in Manual mode

Figure 4-1 Start page on web user interface

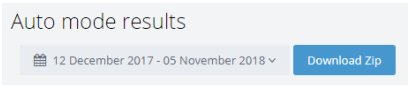
①	Menu	②	State of the device
③	**Latest results**	④	**Language:** Drop-down menu for changing the language.
⑤	Logout	⑥	Progress of the current measurement

4.4 Export an FCS-file

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Go to the page **Results**.	
2.	Find the result in the table.	
3.	Click on the **fcs** link (Position X).	
4.	The download should start.	

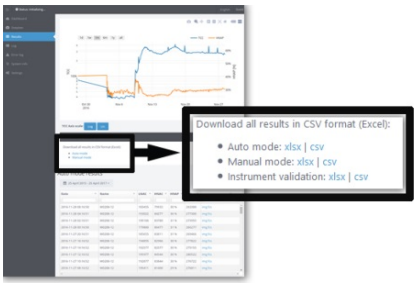
4.5 Download a zip file with multiple results

The results from multiple measurements can be downloaded as a zip file. This zip includes the FCS file and summary plots of each measurement.

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Go to the page **Results**.	
2.	Find the results for the mode you are interested in: **Auto** or **Manual mode** results.	
3.	Select the date range you want and click **Download Zip**. A progress bar will appear at the top of the browser until the zip starts downloading.	
4.	The download starts.	

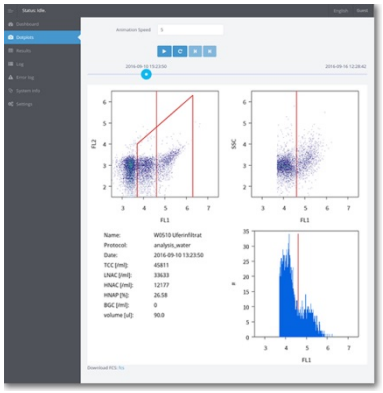
4.6 Export a CSV or XLSX file

The list of all results can be downloaded from the web interface in either Excel XLSX or in CSV format. The **Auto mode** results are separated from **Manual mode** results.

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Go to the page **Results**.	
2.	Find the **DOWNLOAD RESULTS** box, below the graph.	
3.	Click on the requested file.	
4.	The download starts.	

4.7 Animate the evolution of dot plots

The web interface can animate dot plots from the **Auto mode**, to help visualize the progression of the measurements. This feature shows static images in rapid succession; therefore the animation can only be exported using screen recording software.

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Go to the page **Dotplots**.	
2.	Use the previous and next arrows or the slider to centre the slider on the desired date range.	
3.	Press **Play** to start the animation.	
4.	Adjust the animation speed as needed in the field above (animation speed).	

4.8 Change the measurement interval

If the instrument is in **Auto mode**, the measurement interval can be changed from the web interface:

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Go to the page **Settings**.	
2.	Change the duration of the measurement interval (Position X).	
3.	Click **Save** to confirm.	

4.9 Take screenshots of the touch GUI

Take screenshots of the touch GUI from the web interface:

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Press the **System info** button.	
2.	Press **Get Screenshot**.	
3.	The screenshot is displayed below.	

4.10 Power off and reboot from web GUI

The device can be switched off or rebooted from the web interface according to the followed procedure:

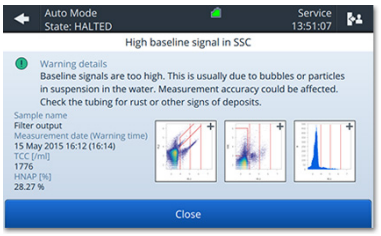
The instrument cannot switch on remotely.

	WORK STEP	ADDITIONAL INFO / IMAGES
1.	Choose the dropdown menu **Sign Out** in the top right corner on the web interface.	
2.	Press **Reboot** or **Power off**.	
3.	The instrument restarts or powers off.	

5 Error messages and troubleshooting

5.1 Warnings

Warnings appear when unusual behavior is detected during an otherwise successful measurement. They can indicate reduced accuracy of the measurement results or indicate impending errors. Unlike errors, warnings do not prevent the instrument from functioning, but operators should pay attention to them as they can indicate sources of inaccuracies.

WARNING	
When unusual behaviour is detected, a warning is shown next to the measurement results. More information on the warning can be found by clicking on the corresponding warning entry next to the measurement results or directly in the warning log.	

The following warning messages can be displayed:

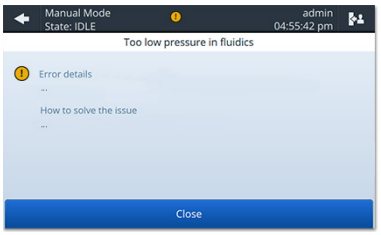
Table 5-1 Warnings

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
W01	Cartridge expired	Cartridge reagents have expired. Measurement accuracy could be affected.	• Please replace the cartridge as soon as possible.
W02-04	High baseline signal in FL1 / FL2 / SSC	Baseline signals are too high. This is usually due to bubbles or particles in suspension in the sample. Measurement accuracy could be affected.	• Check the inlet tubing for any signs of deposits. • Check the water connections, be sure that they are all tight (no air entry). • If the warning persists, please perform cleaning of instrument with the cleaning kit.
W05	Incubator temperature off target	Temperature during incubation was more than 2 °C off target. Cell counts may be underestimated. The incubator may be defective.	• If the problem persists, please contact customer service.
W07	TCC out of range	TCC is above the specification limit of BactoSense of 2’000’000 cells/ml.	• Please dilute the sample to ensure accuracy of the measurement.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
W08	Air bubbles detected	The sample appears to contain air bubbles.	• Check sampling device is installed properly. • Check the sample for bubbles. • If using online sampling device, check water is supplied at correct pressure.
W09	Tray temperature off target	The autosampler tray was more than 2° C off target before the analysis.	• Check whether the insulating cover is in place • Recover and wait until the device has cooled the unit • If the problem persists, the cooling unit may be defective.
W10	Enclosure too cold	Enclosure is too cold, rapid heating has been activated before continuing protocol.	• Instrument cannot safely operate if internal temperature is too cold. • Rapid heating attempts to heat the enclosure to ensure safe operations. • Measurements will start once internal temperature is sufficient.
W11	Service required	Service of the instrument is required. Measurement precision can no longer be guaranteed. **Continued operation can lead to leaks, which can damage the instrument.**	• Next service date is overdue. • Valve and plunger have reached end of life. • Please contact your service representative.

5.2 Non-critical error messages

Non-critical errors prevent a measurement from terminating successfully, but do not prevent the instrument from running another measurement afterwards. These errors do not require human intervention. Some non-critical errors are promoted to critical errors if they repeat three times.

NON-CRITICAL ERRORS	
The protocol stops. The cause of the error is usually fixed by repeating the analysis or waiting. • Another measurement can immediately be started. If it completes successfully, the error state is cleared. • More information on the error can be found in the error log, by clicking on the corresponding error entry.	

The following non-critical error messages can be displayed:

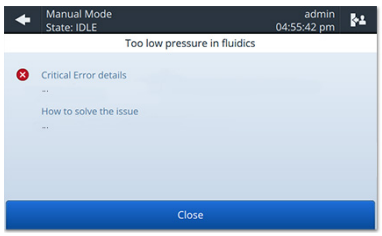
Table 5-2 List of non-critical errors.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E01	Cartridge door open	Cartridge door is open and prevents measurements from running.	• Close door and retry the measurement.
E08	Enclosure too damp	Enclosure humidity is too high for safe operation.	• Replace desiccant bag, by unscrewing the large cap on the left-hand-side of the instrument (Instruction Manual). • If the problem persists, please contact customer service.
E09	System overheated	Inside temperature is too high for safe operation.	• Reduce ambient temperature or increase measurement interval to allow instrument to cool down.
E14	Laser too hot	Laser temperature is too high for safe operation.	• Reduce ambient temperature or increase measurement interval to allow instrument to cool down.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E15	Mixer inflation error	Underpressure detected during mix or dispense.	• Missing sample. • Air in the input. • Leak in the system. • Please execute **Clean Optics** protocol.
E18	Pump underpressure error	Underpressure detected during dispense.	• Missing sample. • Air in the input. • Leak in the system. • Please execute **Clean Optics** protocol.
E19	System overheated	The inner temperature is too high for operation.	• Reduce ambient temperature, or increase measurement interval to allow instrument to cool down.
E33	Processing memory overflow	Signal processing circuits memory has overflown.	• Retry the measurement. Ideally execute a **Clean Optics** protocol first.
E42	Enclosure too cold despite heating	Enclosure temperature remains too cold after heating procedure.	• Ambient temperature is less than 5 °C. • Increase ambient temperature.
E44	Pump module startup error	The pump module failed to power on correctly.	• Simply retry the measurement.
E45	Low valid volume ratio error	The valid volume ratio is too low. This can be caused by a too elevated bacterial content in the water or turbidity/debris.	• The accuracy of the measurement is probably very low. Repeat the measurement while diluting the sample with clear water.
E46	Cartridge is still busy	The cartridge was still initializing when the measurement started.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.
E48	Microfluidic subsystem busy	The microfluidic controller is performing another task while the measurement was taken.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E49	GPIO Timeout error	The processing board is waiting for another module to respond.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.
E50	Pump motion blocked	The motion of the pump is blocked.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.
E51	Unable to get temperature reading	Unable to get temperature reading. The control loop is thus disabled.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.
E52	Cartridge valve motion blocked	The motion of the cartridge valve is blocked.	• Non-critical error. Repeat the measurement. If the problem persists, contact a service technician.

5.3 Critical Errors

CRITICAL ERRORS	
If a **critical error** occurs during operation, it has the following effects: • The protocol immediately stops. • The instrument goes into critical error state, and manual intervention is needed before any new protocol can be launched. • The cause of the error must be solved by an operator, then the errors can be manually cleared from the **Error Log.**	

The following critical error messages can be displayed:

Table 5-3 List of critical errors.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E00	General alarm	Unhandled error.	• Please contact customer service
E02	Cartridge empty	Cartridge is empty.	• Replace cartridge (Instruction Manual)
E04	Cartridge missing	Cartridge is missing.	• Insert a cartridge (Instruction Manual)
E05	Full waste bag	Cartridge waste bag is full.	• Replace cartridge (Instruction Manual)
E06	Cartridge communication impossible	Cartridge is disconnected, door is open, or cartridge electronics are damaged.	• Make sure the cartridge's electronic cable is connected properly, and that the door is properly closed. • If that doesn't help, replace the cartridge and inform customer service.
E07	Storage disk full	Insufficient storage space to continue operation.	• Delete old measurements and try again (Instruction Manual) • This will delete the measurements permanently from the device. • Export measurements as backup solution.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E12	FPGA initialization error	Failed to initialize the signal processing chip.	• Reboot the instrument.
E13	Laser end of life	Laser has reached end of life.	• Service required.
E16	Processing error	An error was discovered while processing the signals.	• The accuracy of the latest result is not guaranteed. Please repeat the analysis.
E17	Overpressure detected	Overpressure detected during dispense.	• Likely cause: The output filter is probably clogged. • Optical flow cell blocked. • Please call customer service.
E20	Abnormal shutdown	Protocol interrupted for unknown reasons, usually due to a power cut.	• Usually indicates a power cut, or other external interruption to the measurement. • Perform a **Clean Optics** and **Clean Sampling Device** protocol before starting a new measurement.
E23	Temperature Sensor Communication Error	Communication with temperature sensor on IO board failed.	• Service required.
E24	Laser Communication Error	Communication with optical unit failed.	• Service required.
E25	Pump Communication Error	Communication with pump module impossible.	• Service required.
E26	Power Supply Communication Error	Communication with power supply module impossible.	• Service required.
E27	Pump Communication timeout	Communication with pump module timed out.	• The cartridge door is open. • A cable is disconnected. • A circuit board is damaged, either in the pump or cartridge.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E28	Empty dye supply	Dye tube connected to cartridge is empty.	• The tube is disconnected. • Cartridge is defective. • Repeat the cartridge change procedure, making sure that all tubes are properly connected.
E29	Empty rinse fluid supply	Rinse fluid tube connected to cartridge is empty.	• The tube is disconnected. • Cartridge is defective. • Repeat the cartridge change procedure, making sure that all tubes are properly connected.
E30	Empty bleach supply	Bleach tube connected to cartridge is empty	• The tube is disconnected. • Cartridge is defective. • Repeat the cartridge change procedure, making sure that all tubes are properly connected.
E31	Cartridge electronics failure	Cartridge level can no longer be determined accurately.	• Replace cartridge and contact customer service.
E32	Incompatible cartridge	Cartridge type is incompatible with this instrument or software.	• Make sure that you have the correct cartridge type for this instrument.
E33	Processing memory overflow	Signal processing circuits memory has overflowed.	• Run the **Clean optics** protocol and retry the measurement
E34	Needle Arm Position Invalid	Autosampler needle position error: Autosampler needle is at an unexpected location.	• Repeat the protocol. Likely causes: the last protocol was interrupted abruptly.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E35	No Tray Detected Error	No vial detected	• Check that the tray and vials are installed in the autosampler. • Place a tray in the autosampler, then clear the error and retry. • If the tray is present or the carrier is stuck, power the autosampler off and on again.
E36	Temperature Wait Timeout	The tray failed to reach its target temperature after one hour.	• Make sure the insulating cover is in place, clear the error, and retry. • If the problem persists, the cooling unit may be defective.
E37	Autosampler Communication Error	Communication with autosampler impossible.	• Make sure the autosampler is powered on and connected to the instrument. Clear errors, and try again.
E38	Cartridge not ready error	The new cartridge has not been initialized correctly	• Execute the "Cartridge Change" procedure (Instruction Manual)
E39	Laser current limit reached	The laser has reached its upper current limit; indicating end of life.	• The laser needs to be replaced. Contact a service technician.
E40	Set of gates not found	Reference to the set of gates selected for the measurement cannot be found in the instrument settings.	• The set of gates has been deleted. • Change the set of gates and retry the measurement.
E41	Set of gates incompatible	The set of gates selected for the measurement cannot be used with this cartridge.	• Correct cartridge change procedure was not followed. • Incorrect digital input configuration. • Change the set of gates and retry the measurement.

ERROR CODE	NAME	DESCRIPTION	CAUSES / WHAT TO DO
E43	External temperature too low	Ambient temperature is outside of the instrument specifications.	• Increase ambient temperature. • Trying to operate or store the instrument below 5 °C can cause damage to the device.
E47	Unable to find main reference magnet during homing	During initialization, the valve did not home properly.	• Service required.
E53	Unable to communicate with motor	The communication to the motor controller of the mixer is defective.	• Service required.

5.4 Low temperature operation and standby heating

The instrument needs to operate in a controlled temperature range to ensure reliable and reproducible measurement results. At very low temperatures and very high ambient temperatures (below 5 °C or above 30 °C), protocols are forbidden from running. At temperatures between 5 °C and 20 °C, the instrument regulates the enclosure temperature in two ways:

Standby heating is activated between protocols to maintain a sufficiently enclosure temperature. It is automatically powered off after 12 hours. In the top bar a little temperature freezing sign appears when the standby heating is on.

Rapid heating is activated at the beginning of a protocol. Once the internal temperature is warm enough, the protocol is allowed to execute. The instrument status switches to “Heating...” during this phase and a warning is attached to the measurement (table 5-1, W10). If after several hours the instrument cannot heat itself enough to ensure an accurate measurement, the protocol exits with an error (table 5-2, E42).

WARNING!

Measurements are delayed by rapid heating.

- To avoid this, keep the instrument temperature above 10 °C.

6 Table of acronyms

Common acronyms in flow cytometry are listed below

Table 6-1 Acronyms

NAME OF ACRONYM	DESCRIPTION
FCS	Flow Cytometry Standard (FCS) is a data file standard for the reading and writing of data from flow cytometry experiments. The FCS specification has traditionally been developed and maintained by the International Society for Advancement of Cytometry (ISAC). FCS used to be the only widely adopted file format in flow cytometry.
FL1	Fluorescence Signal 1 (535 nm).
FL2	Fluorescence Signal 2 (715 nm)
GUI	Graphical User Interface.
HNA	High Nucleic Acid = Bacteria with a large amount of DNA which produce a strong fluorescence emission. They are generally regarded as the active part of a microbial community.
HNAC	High Nucleic Acid Count. The number of HNA bacteria inside the TCC or ICC gate, and above the HNA / LNA limit.
HNAP	High Nucleic Acid Percentage = The percentage of HNA bacteria relative to the cell count (HNAC / TCC for TCC cartridge, HNAC / ICC for ICC cartridge)
ICC	Intact Cell Count = Total number of intact bacteria inside of the ICC gate.
LNA	Low Nucleic Acid = Bacteria with a smaller amount of DNA which produce a weaker fluorescence emission than HNA bacteria.
LNAC	Low Nucleic Acid Count. The number of LNA bacteria inside the TCC or ICC gate below the HNA / LNA limit.
SSC	Side Scatter Signal. Scattered light, increases with the size of the detected object or its surface complexity/geometry.
TCC	Total Cell Count = Total number of bacteria detected inside the TCC gate.

7 Index

bNovate Technologies SA
Ch. Dent d'Oche 1A
CH-1024 Ecublens
Switzerland

Tel. +41 (0)21 552 14 21
info@bnovate.com
www.bnovate.com