BACTcontrol microLAN

User Manual

Version: V0.4.4
Date: 01.11.2020

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1.1 VERSION OVERVIEW

Table 1 Version overview

Version	Release date	Changes
V0.4.1	30-05-2016	Basic version
V0.4.1	21-09-2016	Preliminary version
V0.4.2	25-01-2017	Preliminary version
V0.4.3	16-09-2019	Draft version
V0.4.4	28-10-2020	Updated

2. GENERAL

2.1 SAFETY

Figure 1 Wash hands

Water supply can be contaminated with bacteria!
Always wash your hands before and after working with the BACTcontrol.

Figure 2 Caution, risk of electric shock

Figure 3 Hot surface

Always switch off the instrument during maintenance and cleaning.

2.2 COPYRIGHT

2.3 WARRANTY

microLAN b.v. warrants each Model BACTcontrol and its optional equipment against defects in materials and workmanship under normal use and service for a period of one (1) year. Equipment installed by microLAN is warranted from the installation date; all other equipment is warranted from the ship date. If purchaser schedules or delays installation more than 90 days after delivery, then the warranty period starts on the 91st day from date of shipment. This warranty extends only to the original purchaser. microLAN will, at its option, repair or replace equipment that proves to be defective during the warranty period, provided the equipment is returned to microLAN at the expense of the purchaser. Parts, labour, and return shipment to the customer shall be at the expense of microLAN. Travel costs shall be at the cost of the purchaser.

Software and firmware designed by microLAN for use with an external PC will execute its programming instructions when properly installed on that PC. microLAN does not warrant that the operation of the PC, software, or firmware will be uninterrupted or error-free.

Consumables and parts are warranted for 30 days (parts only) and are not available for coverage under extended warranties or service contracts.

This warranty shall not apply to defects originating from:

- Improper maintenance or operation by purchaser.
- Purchaser-supplied accessories or consumables.
- Modification or misuse by purchaser.
- Operation outside of the environmental and electrical product specifications.
- Improper or inadequate site preparation.
- Purchaser-induced contamination or leaks.

2.4 MANUAL INFORMATION

2.4.1 NOTES, CAUTIONS AND WARNINGS

This manual contains Notes, Cautions and Warnings, for situations that may:

- Endanger operating personnel,
- Cause damage to equipment
- Need specific attention.

The following formats and symbols are used:

NOTE

Notes provide additional information, such as expanded explanations, hints or reminders.

CAUTION

Cautions alert you to conditions that may cause damage to the equipment or interfere with the normal processing and damage the product.

WARNING

Warnings point out procedures you must follow precisely to avoid personal injury or serious damage to the equipment.

2.5 ISO 15839

ISO 15839 describes the procedure and performance of on-line sensors/analyzing equipment for water. This standard is applicable to most sensors/analyzing equipment.

This International Standard :

- Defines an on-line sensor/analyzing equipment for water quality measurements;
- Defines terminology describing the performance characteristics of on-line sensors/analyzing equipment;
- Specifies the test procedures (for laboratory and field) to be used to evaluate the performance characteristics of on-line sensors/analyzing equipment.

2.6 RELATED DOCUMENTS

🕮 BACTcontrol Software Manual
🕮 BACTcontrol Advanced user Manual
🕮 BACTcontrol Quick user Manual

📖 Manual Optional Cooling-Unit

3. INTRODUCTION OF THE BACTCONTROL

The determination of the presence of microorganisms is a key parameter in order to determine whether a water body is safe, yet the standard determination methods that are used are based on the cultivation of these microorganisms. These methods are characterized of being time consuming (24-48) and are not suitable for a fast water quality assessment (REF. 3). Due to these time consuming procedures, there is an considerable chance that populations get exposed to microbiological hazards, before the results of the tests are known (REF. 1). Microbiological contamination is a constant concern in a wide range of fields. For example water treatment, food industries and pharmaceutical companies are fields where fast detection of dangerous microorganisms that might cause diseases is extremely important. Diseases that are present in water bodies can be prevented by monitoring (REF. 1). Enzymatic activity determination is one detection process that is suitable for rapid measurements of dangerous microorganisms (REF. 3). Unfortunately there is still a significant lack of automated on-line systems for the detection of microbial parameters, which speed up the detection process of dangerous microorganisms (REF. 3).

The BACTcontrol is an on-line automated instrument for the detection of microbiological activity in water. It measures the specific enzymatic activities of β-galactosidase (coliforms), β-glucoronidase (E.coli), ß-glucosidase (Enterococci) and alkaline phosphatase (Total Activity, biomass), as an indicator for the presence of bacterial contamination. The enzyme activity is detected by adding reagents (consumables) which contain a fluorescent indicator. The reagents are substrate specific for the enzyme to be detected, meaning that fluorescence will increase when the corresponding enzyme is present in the sample.

Figure 4 BACTcontrol

The BACTcontrol is an “Early warning system”, complementing the officially accepted methods for the detection of microbiological activity. The measurements are realized in a short period of time (1-2 hours), in contrast to classical microbiological methods, which are labour intensive and in which cultivation of the organisms is required, taking several hours before getting reliable results (24-48).
In Appendix B a more detailed explanation about enzymes and the working of the BACTcontrol is given.

4. TECHNICAL DETAILS

4.1 CONDITIONS

The BACTcontrol is specially designed for use on a table or on a trestle.

Place the BACTcontrol in a properly ventilated area

Make sure the BACTcontrol setup is at least 20 cm from a wall

The humidity conditions in the area should be between 20 and 80 %

Keep the BACTcontrol out of direct solar irradiation

Protect the BACTcontrol from rain

The area temperature should be between 15– 35°C / 59 – 86°F. In case of higher temperatures, an optional Cooling-Unit is recommended (Part nr: 01BACT001A516)

Figure 5 Optional enclosure Cooling-Unit (Part nr: 01BACT001A516)

4.2 MATERIAL AND WEIGHT

Cabinet: Stainless steel 316

Protection classification: IP 54 (IP 65 optional)

Weight : 25kg (excl. optional cooling-unit 30kg in total)

4.3 FLOOR SPACE BACTCONTROL

Depth: 321 mm (347 mm incl. hinges & door handle)

Width: 450 mm (482 mm incl. connectors)

Height: 460 mm (470 mm incl. rubber feet)

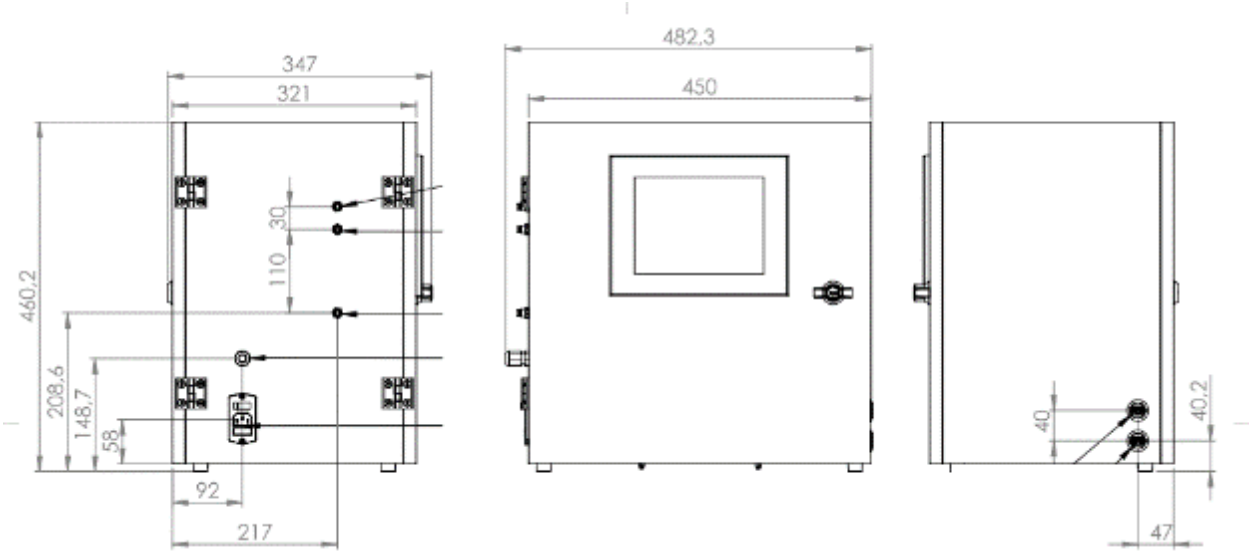


Figure 6 Dimensions BACTcontrol

4.4 SAMPLE DETAIL

Sample inlet

- Sample connection: 4 mm ID
- Tube size: 2,5 mm ID x 4,0 mm OD
- Sample pressure: max 0.05 bar
- Sample flow: 3l/h
- Sample temperature: 10- 35°C / 50 – 95°F

Second inlet/ Extra rinsing

- Sample connection: 4 mm ID
- Tube size: 2,5 mm ID x 4,0 mm OD
- Sample pressure: max 0.05 bar
- Sample flow: 3l/h

Sample temperature: 10- 35°C / 50 – 95°F

Sample outlet and waste

- Sample connection: 4 mm ID

- Tube size: 2,5 mm ID x 4,0 mm OD
- Sample flow: 3l/h

4.5 ELECTRICAL CONDITIONS

Operation voltage: 230V - 50Hz or 110V – 60Hz

Nominal power consumption: <50W (without optional cooling unit)

Peak power consumption: <700W (included optional cooling-unit)

It is recommended to protect the electrical supply with an earth leakage circuit breaker

4.6 ENVIRONMENTAL CONDITIONS

Place the BACTcontrol in a properly ventilated area

Temperature between 15– 30°C / 59 – 86°F, (at higher temperatures, an optional Cooling-unit is recommended)

No direct solar irradiation

Protect from rain

Humidity conditions between 20 and 80 %

4.7 SPECIFICATIONS OPTIONS

Modem slot for UMTS, ISDN or analog (modem optional)

Second sample inlet / extra rinsing

Cooling-Unit (if higher than 30°C / 86°F)

4.8 GENERAL COMPUTER SPECIFICATIONS

Integrated PC with Windows professional SP1 operating system

Graphical user interface with touchscreen

Touchscreen power DC12V/5A

4.9 SYSTEM SPECIFICATIONS

Processor: Onboard Intel® Cedarview dual core D2550 (1.86GHz,1MB L2 cache, 10W) mobile Processor

Chipset: Intel® NM10 Chipset

Ram: Onboard 2GB DDR3 1066MHz Memory

Graphics: Intel CedarView Integrated Graphics Engine with GMA3650

IP65: YES

Power: DC12V/5A Power Adapter

2 x USB 2.0 type A

2 x LAN 10/100/1000MB/s; RJ-45

1 x DB-9 RS-232/422/485 COM1; default RS-232

1 x DB-9 RS-232; COM2

Protocols: Modbus, others on request

1x 4 - 20mA output

Operating system: English

4.10 SCREEN

Integrated PC with Windows

Graphical user interface with touchscreen

LCD Size: 8,4 inch

Display type: XGA LCD

Resolution: 800 x 600

Display color: 262K

Viewing Angle: 65/65/65/45

Brightness: 300cd / C m2

Contrast: 500:1

Touchscreen: Five-wire resistive touch screen

4.11 CONNECTIONS TOUCH PANEL

Figure 7 Connections touch panel

1 On/Off button

2 Com port (COM1)

3 USB ports (4)
(one port is used for software log, other three ports are available for external use)

4 Network connections (2)

5 Com port (COM2)

6 Power input DC12V/5A

4.12 COMMUNICATION SPECIFICATIONS

Full network capability via direct LAN

Data output:

- Digital: Modbus
- Analog: 1x 4 - 20mA output

5. MEASUREMENT SPECIFICATIONS

Table 2 Measurement specifications

Parameter	**E. coli**		**Coliform**		**Total activity (TA)**		**Enterococci**	
Temperature measurement	44,0 °C 111,2 °F		36,0 °C 96,8 °F		45,0 °C 113,0 °F		37,0 °C 98,6 °F	
Reactor chamber size	**2ml**	**10ml**	**2ml**	**10ml**	**2ml**	**10ml**	**2ml**	**10ml**
Buffer dosing [µL]	60	250	60	250	-	-	60	250
Reagent dosing [µL]	60	250	120	500	60	250	60	250
pH	6,9		6,9		10,0		8,8	
Stabilization time [min]	20		20		20		20	
Incubation time [min]	20		20		20		20	
Enzyme	ß-glucuronidase		ß-galactosidase		alkaline phosphatase		ß-glucosidase	
Substrate fluorescent compound	4-Methylumbelliferyl β-D-glucuronide		4-Methylumbelliferyl ß-D-galactopyranoside		4-Methylumbelliferyl phosphate		4-Methylumbelliferyl ß-D-glucopyranoside	

5.1 THE ANALYZER

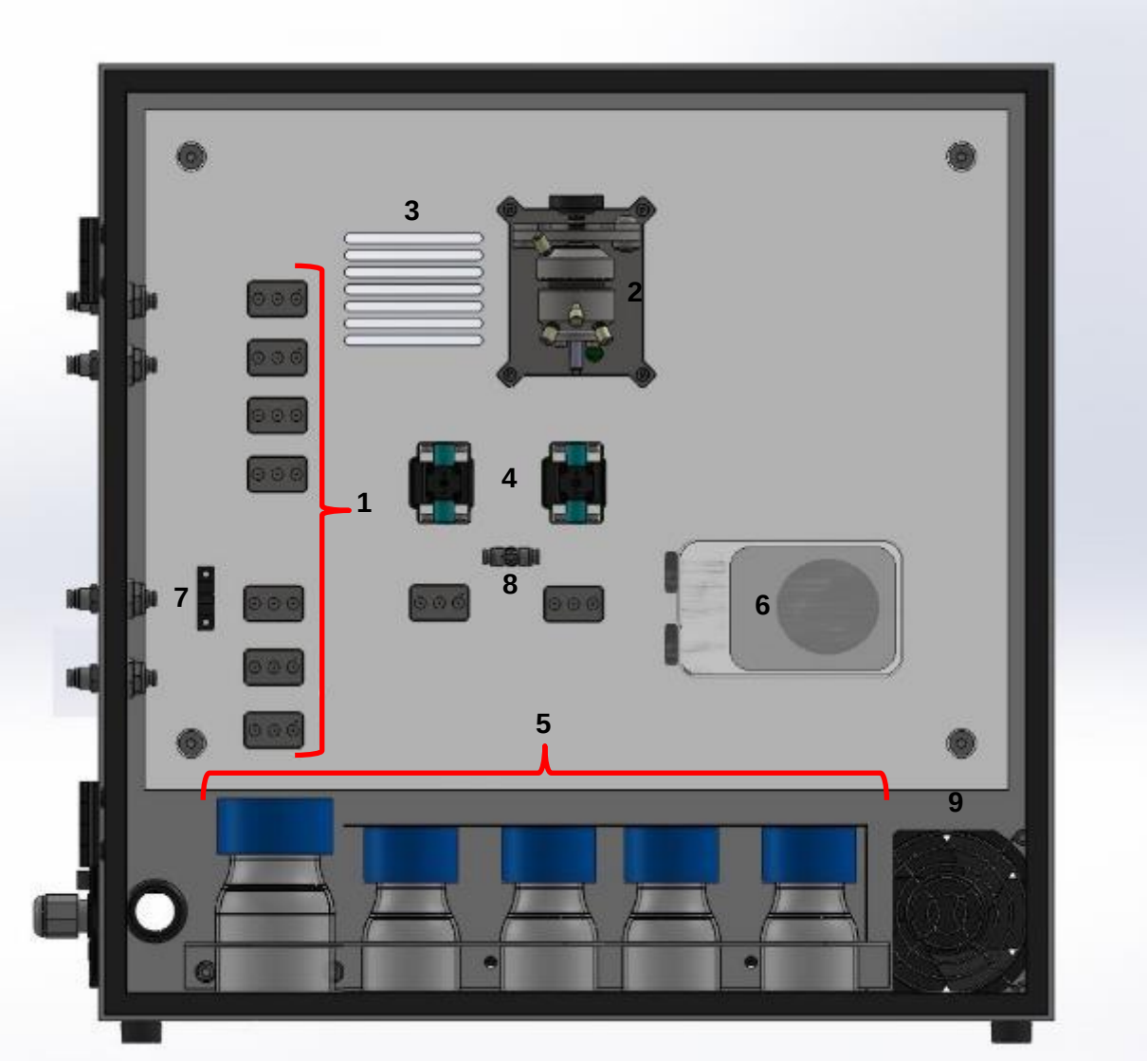


Figure 8 Overview inside BACTcontrol (frontside)

1 Valves
2 Reaction chamber
3 Vent hole
4 Reagent and buffer pumps
5 Cleaning solution, buffer and reagents
6 Sample pump
7 Bubble sensor
8 Pressure senor

9 Ventilator

5.2 REACTION CHAMBER

In the reaction chamber the measurements take place. When the concentration of bacteria in the sample is low, the bacteria has to be concentrated in order to be able to detect the fluorescence and have reliable results. This is done with a porous ceramic filter (Figure 9). The sample is pumped into the reaction chamber and flows through the ceramic filter (size 0,45 µm). The pores of the filter are small enough to retain the bacteria and let the water flow through. When the concentration of bacteria is high enough and there is no need to concentrate it, the dummy filter can be used instead of the ceramic filter. The dummy filter is made from metal, so liquid cannot flow through it. In this case the sample will be pumped to the reaction chamber and a valve is kept open in order to let the first millilitres of sample flow through the outlet, increasing the chance that a representative sample will be measured. When the chamber is filled the measurement takes place.

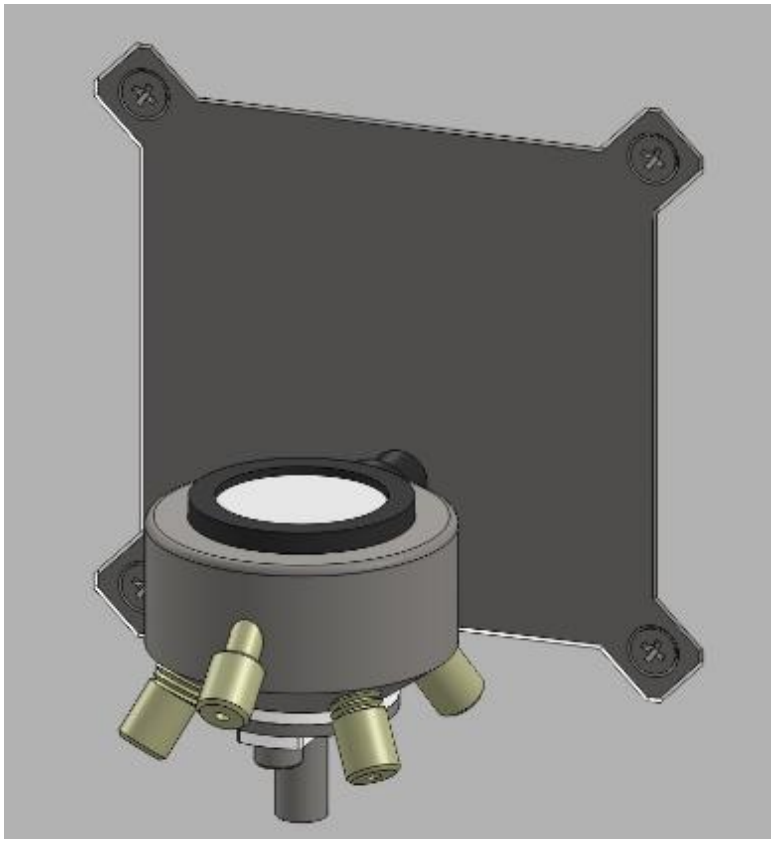


Figure 9 Reaction chamber with ceramic filter with C-ring

After the sample has been heated up to the right temperature, the photodiode emits light with a wavelength of 366 nm through the photodiode window into the reaction chamber (Figure 10). As the bacteria to be determined is present in the sample, the hydrolysed MU substrate will fluorescence (APPENDIX A: GENERAL WORKING OF THE BACTCONTROL).
The fluorescence is detected by the photodiode and converted into enzymatic activity and expressed in picomole per minute

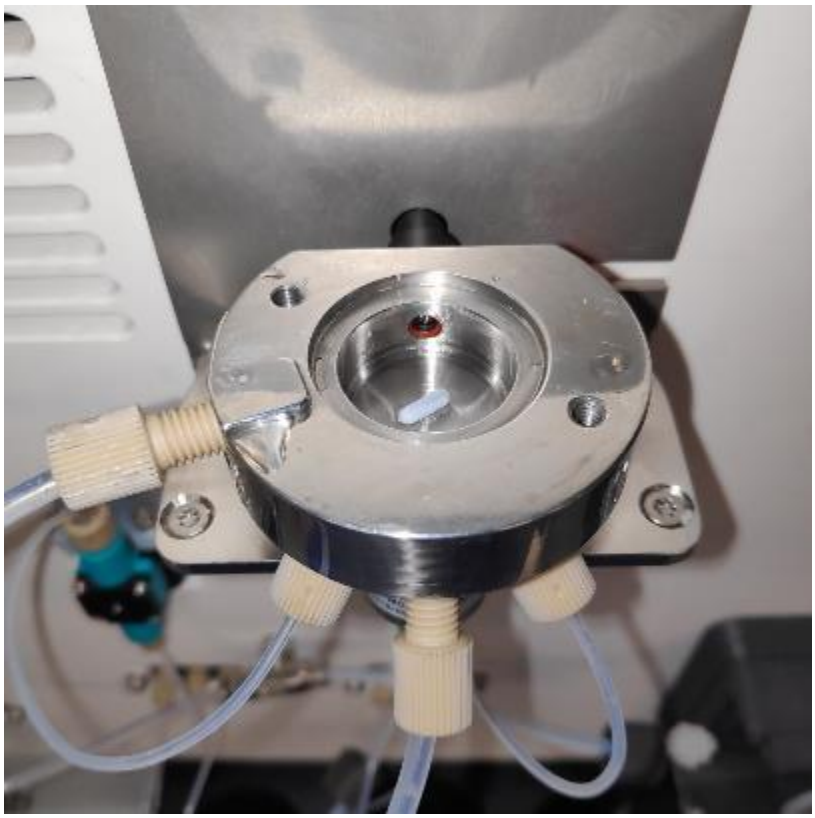


Figure 10 Overview reaction chamber and photodiode window and stirrer

5.3 PHOTODIODE SENSOR UNIT

A photodiode is a light detector, which converts light into voltage or current, depending on how the device is programmed. A photodiode consist of optical filters and it is built-in lenses. The photodiode is responsible for the detection of fluorescence which is an indication of enzymatic activity of specific bacteria (E.coli, coliform, total activity) in the sample. The photodiode is connected to the reaction chamber, where the sample is collected.

5.4 TEMPERATURE SENSOR

The temperature sensor ensures that the measurements are carried out at the correct temperature. Each bacteria has their own temperature optimum, therefore it is important to monitor it. Also in the case of the calibration it is important to have a temperature sensor, since the calibration solution is highly sensitive to temperature changes.

5.5 PRESSURE SENSOR

The pressure sensor is placed between the sampling pump and the reaction chamber and monitors the sample pressure (see figure 8), which should not exceed 3 bar. After a period of time and depending on the type of sample the ceramic filter might get blocked by the many particles present in water, decreasing the sample flow and increasing the sample pressure. When the pressure exceeds the maximum, the system will send a warning and stop the measurements.

5.6 BUBBLE SENSOR

The bubble sensor monitors bubbles flowing from the sample pump into the reaction chamber. Bubbles might cause disturbances or deviating results.
Also a process alarm will be activated in case the water flow of the sample supply is interrupted.

5.7 PUMP UNITS

The BACTcontrol is equipped with two different types of pumps.
(three or four pumps in total, depending on single or multiple measurement).

5.7.1 SAMPLE PUMP

The sample pump is an peristaltic pump without valves and only one moving part, the rotor.

5.7.2 REAGENTS PUMPS

The BACTcontrol uses two or three micro-pumps (depending on single or multiple measurement) to add the reagents in the reaction chamber. The micro-pumps of the BACTcontrol are equipped with a special pump head, which possess an UNF-1/4" thread connection.

5.8 REAGENTS

The Buffer and reagents that can be used in the BACTcontrol:

Figure 11 Reagents overview

1. Cleaning solution concentrate: Contains sodium hypochlorite solution (<0.05% Cl active).
2. Buffer reagent: Contains Tritron-X-100 and MOPSO (β-Hydroxy-4-morpholinepropanesulfonic acid) which is a common buffering agent in biology and biochemistry which is used to keep the pH value stable during a certain period of time.
3. E.coli reagent: Contains the fluorogenic substrate 4-Methylumbelliferyl β-D-glucuronide (MUG) to which ß- glucuronidase enzymes present in E.coli bacteria will attach and will hydrolyse.
4. Enterococci reagent: Contains the fluorogenic substrate 4-Methylumbelliferyl ß-D-glucopyranoside (MUD) to which ß- glucosidase enzymes present in enterococci bacteria will attach and will hydrolyse.
5. Coliform reagent: Contains the fluorogenic substrate 4-Methylumbelliferyl ß-D-galactopyranoside (MUGal) to which ß- galactosidase enzymes present in coliform bacteria will attach to and will hydrolyse.
6. Total activity reagent: Contains the fluorogenic substrate 4-methylumbelliferyl phosphate (MUp) to which alkaline phosphatase enzymes present in all bacteria will attach to and will hydrolyze. Since this reagent contains buffer, no extra buffer reagent will be added when total activity measurement is started. The determination of total activity is an optional measurement. Before starting this measurement, the reagent of total activity should be placed in the BACTcontrol, replacing the E.coli, enterococci or coliform reagents.

5.8.1 EXPLANATION SUBSTRATE

The substrate contained in all three solutions mentioned above is Methylumbelliferone (MU) in combination with another enzyme specific compound. MU is a fluorescent indicator; it is colourless at pH 7.0 and exhibits a blue fluorescence at pH 7.5. The fluorescence intensity of MU is pH dependent and increases to a maximum at pH 10 . The fluorescence at pH 10.3 is approximately 100 times as intense as at pH 7. It is therefore extremely important to add a buffer before each measurement or calibration, so the fluorescence intensity remains stable (Sigma-Aldrich, n.d.)

MU Methylumbelliferone

MUG 4-Methylumbelliferyl β-D-glucuronide

MUD 4-Methylumbelliferyl ß-D-glucopyranoside

MUGal 4-Methylumbelliferyl ß-D-galactopyranoside

MUp 4-Methylumbelliferyl phosphate

rfu relative fluorescence units

5.8.2 STORAGE AND USE OF THE REAGENTS

Table 3 Recommended storage and use of reagents

E.coli reagent	4°C / 39.2 °F Dark place. **CAUTION** (Solid when < 15 °C / 66,4 °F) 1 year storage when unopened. Consumption within 3 months when opened and kept at room temperature.
Enterococci reagent*	4°C / 39.2 °F Dark place. **CAUTION** (Solid when < 15 °C / 66,4 °F) 1 year storage when unopened. Consumption within 3 months when opened and kept at room temperature.
Coliform reagent*	4°C / 39.2 °F Dark place **CAUTION** (Solid when < 15 °C / 66,4 °F) 1 year storage when unopened Consumption within 3 months when opened and kept at room temperature.
TA reagent (Total activity)	-20°C / -4 °F Dark place. 4 months storage when unopened. Consumption within 1 to 2 months when opened and kept at room temperature.
Buffer	-20°C / -4 °F Dark place 1 year storage when unopened Consumption within 1 to 2 months when opened and kept at room temperature.

* **IMPORTANT!**

Storage at -20°C / -4 °F is also possible for E.coli and coliform reagents. However this might affect the solubility of MUG after thawing. In case it is kept at -20°C / -4°F, homogenize thoroughly after thawing.

E.Coli, Enterococci and Coliform reagent can be **SOLID** when < 15 °C / 66,4 °F.
This will not affect the reagent and it will be liquid again after warming up above 15 °C / 66,4 °F.

Longer periods of storage than as described above may lead to an unstable/ nonhomogeneous solution, causing deviating results.

6. BACTCONTROL INSTALLATION

Installation of the BACTcontrol system is performed by engineers of microLAN or qualified engineers of the local distributor.

The procedures in this section should only carried out by authorized engineers.

6.1 CONNECTIONS ON THE BACTCONTROL

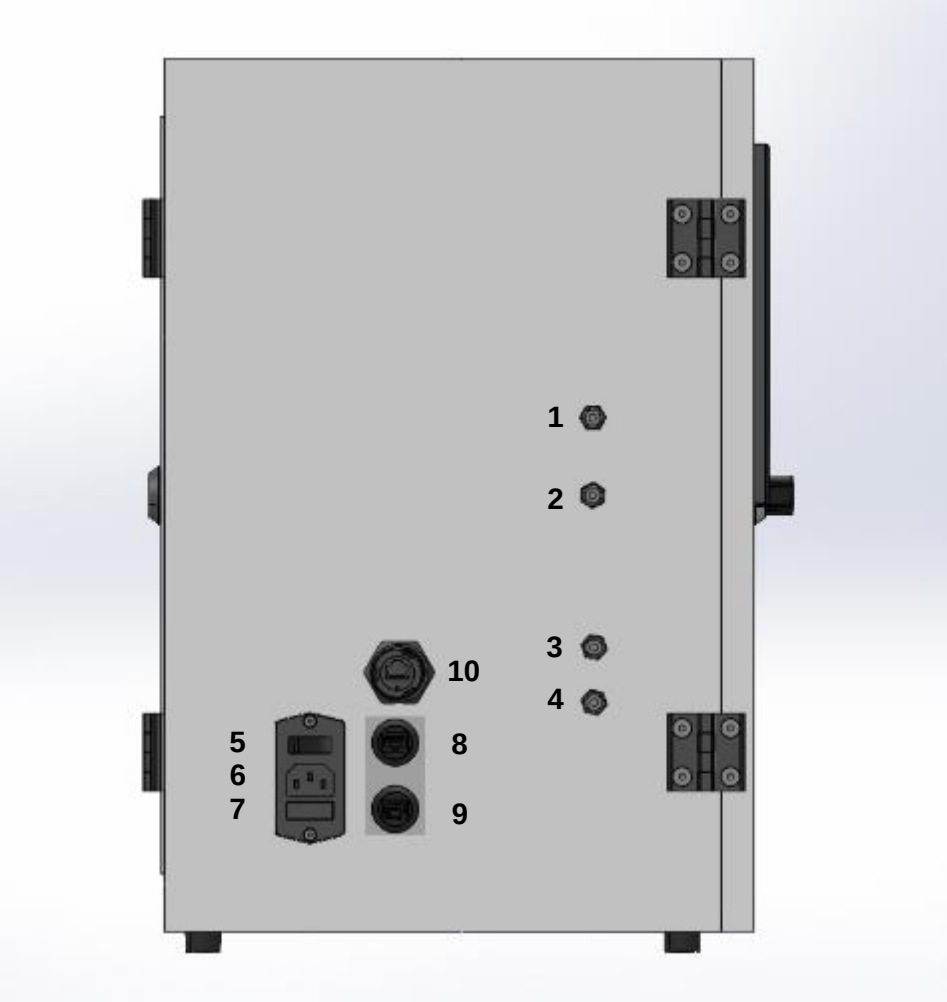


Figure 12 Connections left side and right side BACTcontrol

1 Outlet waste
2 Outlet sample
3 Inlet sample
4 Second inlet/Extra rinsing
5 On/Off Button
6 Power connection main power
7 Fuse box
8 USB type-A input
9 Ethernet RJ-45 input
10 Cable gland for wiring 4-20 mA and alarm relays

6.2 CONNECTION OF THE WATER SAMPLE INLET, OUTLET & WASTE

The waste can be separately connected to a container *.

- Make sure the tubing is pushed completely into the connectors!

Figure 13 installing tubing

* Any disposal practice must be in compliance with all local and national laws and regulations. Customers are advised to check their local legislation governing the disposal of waste materials. If this preparation becomes a waste, the final user must define and assign the appropriate European Waste Catalogue code. Use only authorized contractors

6.3 CONNECTING ALARMS AND ANALOG OUTPUT

Alarm and analogue output are located on the printed circuit board (PCB) in the backside compartment of the BACTcontrol.

- Instrument Alarm: Connect wiring (DC) to **82 (+)** and **83 (-)**

The BACTcontrol has one analogue output (4-20 mA) for connecting to an external controller or system:

- Connect the wiring to 76 (-) and 77 (+)

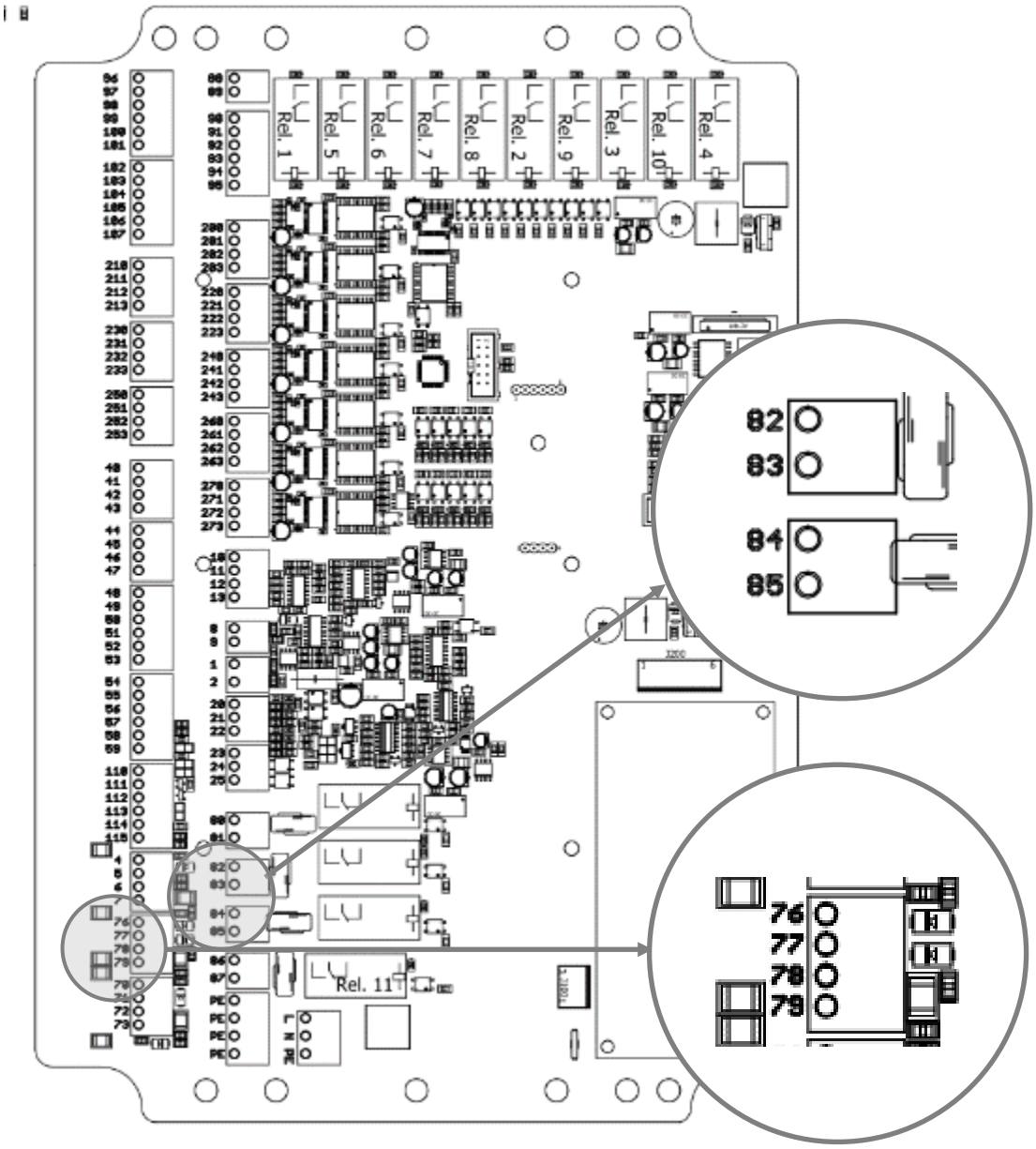


Figure 14 Printed circuit board of the BACTcontrol

6.4 POWER SUPPLY

The power supply cable should be connected on the main power connection (5)

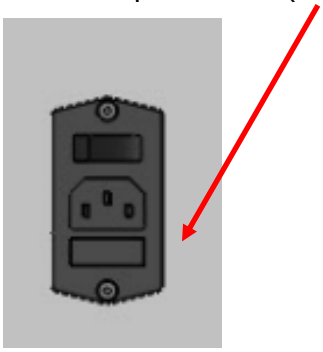
Operation voltage: 230V - 50Hz or 110V – 60Hz
Peak power consumption: <700W

It is recommended to protect the electrical supply with an earth leakage circuit breaker

6.5 CHANGING/REPLACING FUSES

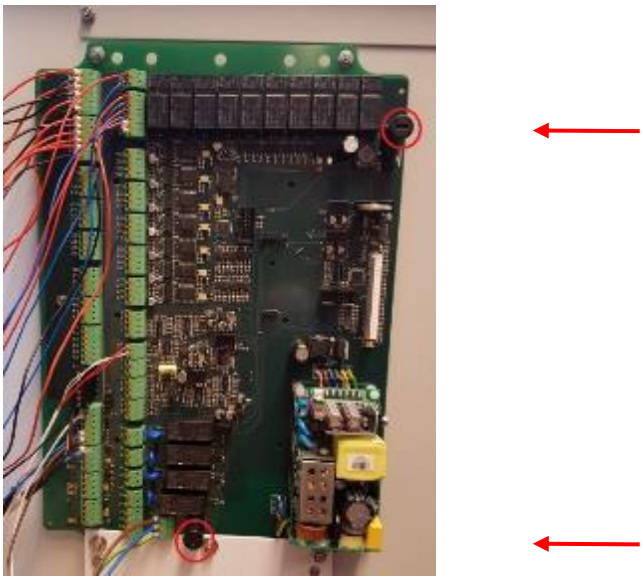
For protection of the electrical system three fuses (part nr. 06BACT001A269) are installed in the BACTcontrol.

1. The main power fuse (Nr 7 left side BACTcontrol)



2. PCB fuses

The printed circuit board is protected with two fuses



7. INTRODUCTION TO THE BACTCONTROL SOFTWARE

For a complete explanation of the software, see: BACTcontrol Software Manual

7.1 LOG IN

The operating terminal is a touchscreen that can be operated through finger contact or with a touchscreen pen. Do not touch the screen of the operating terminal with hard objects, this might damage the terminal.

7.2 STATUS

In the status section, the main procedures can be operated. Details of the measurement and possible alarms are displayed. Figure 15 shows the different options that are available in the status screen together with the information of the current or last measurement.

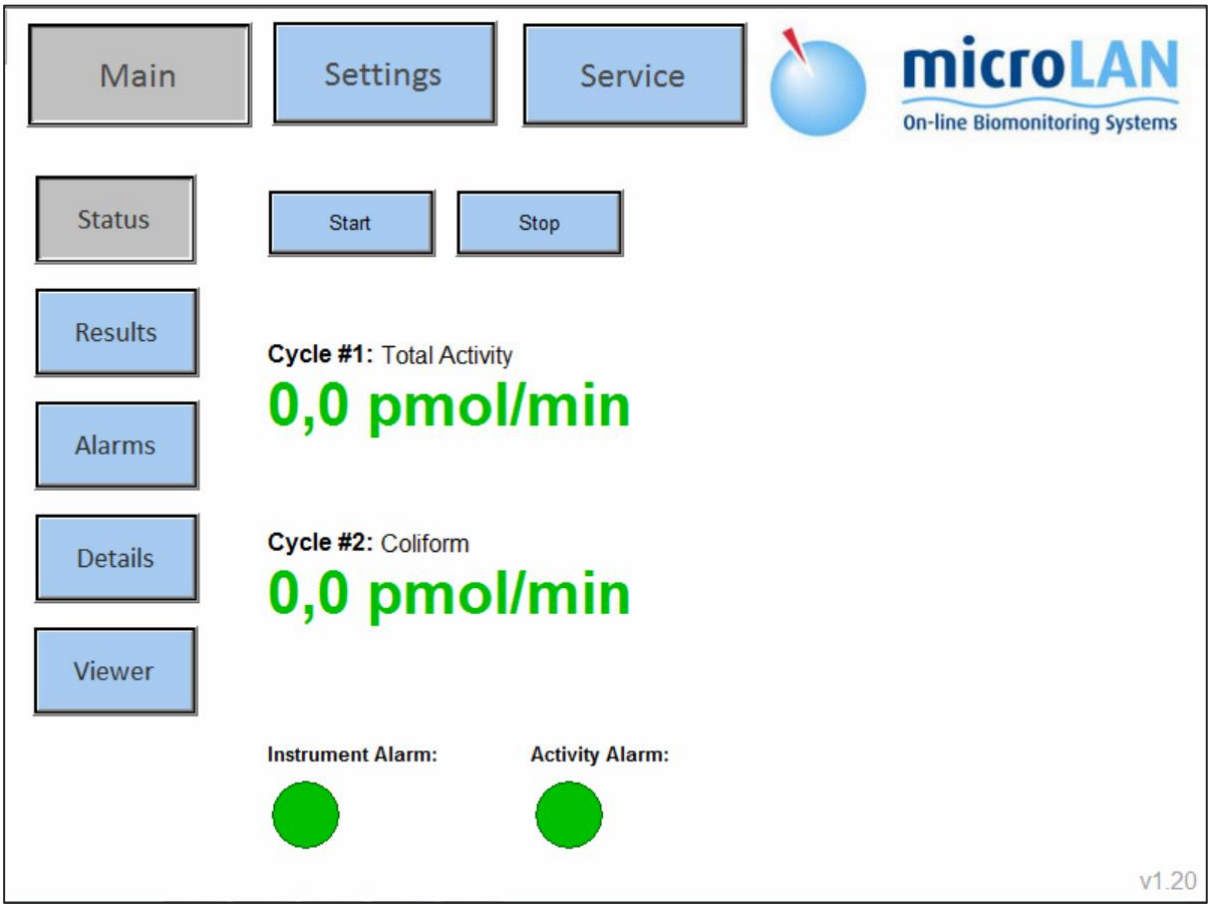
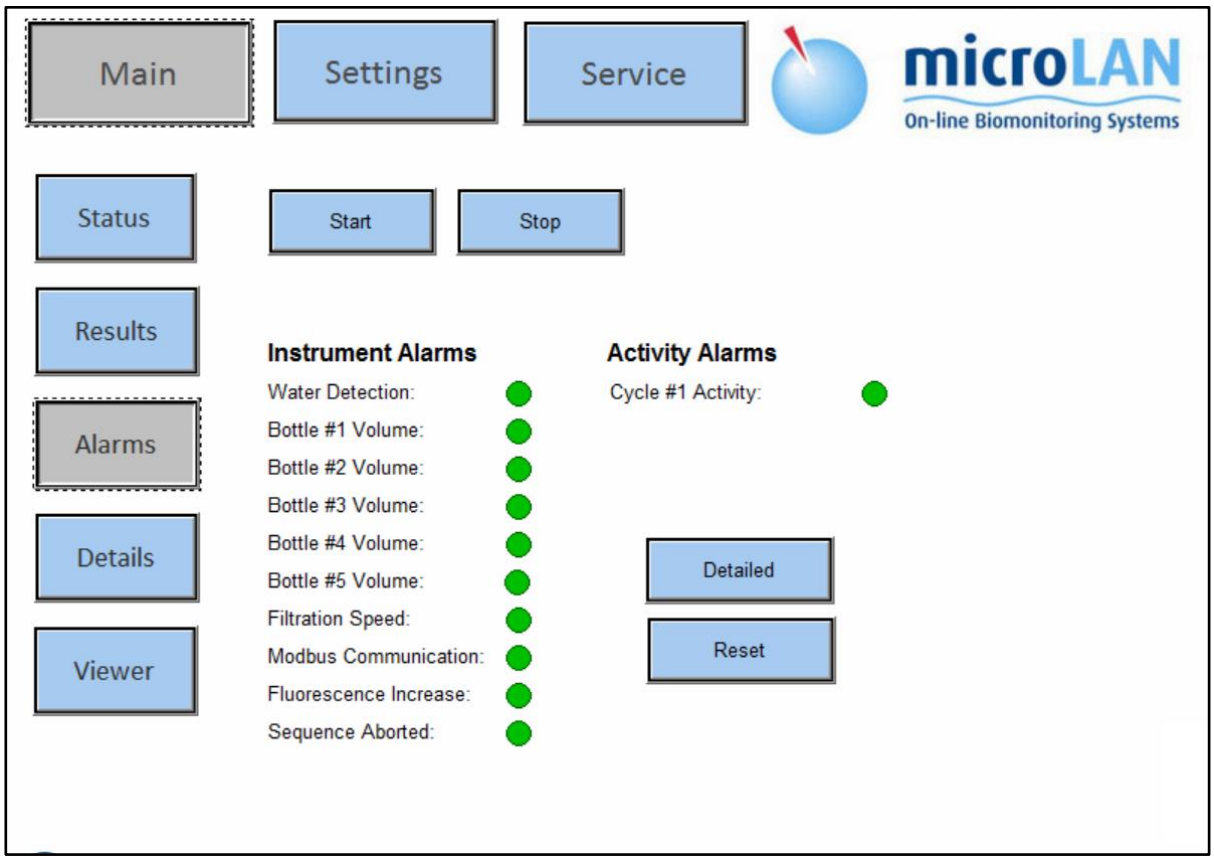


Figure 15 Status screen

7.3 ALARMS

In this screen alarms are displayed.

For example: Erroneous measurement when fluorescence increase is to low or when detection limit is higher than measuring result (only above 5 pmol)



7.4 DETAILS

Process details such as temperature, volume of solutions, status of valves and pumps are displayed in Details section.

When a solution should be replaced an instrument alarm will appear. For instructions regarding the replacement of solutions see chapter 8.1.2 (Daily maintenance)

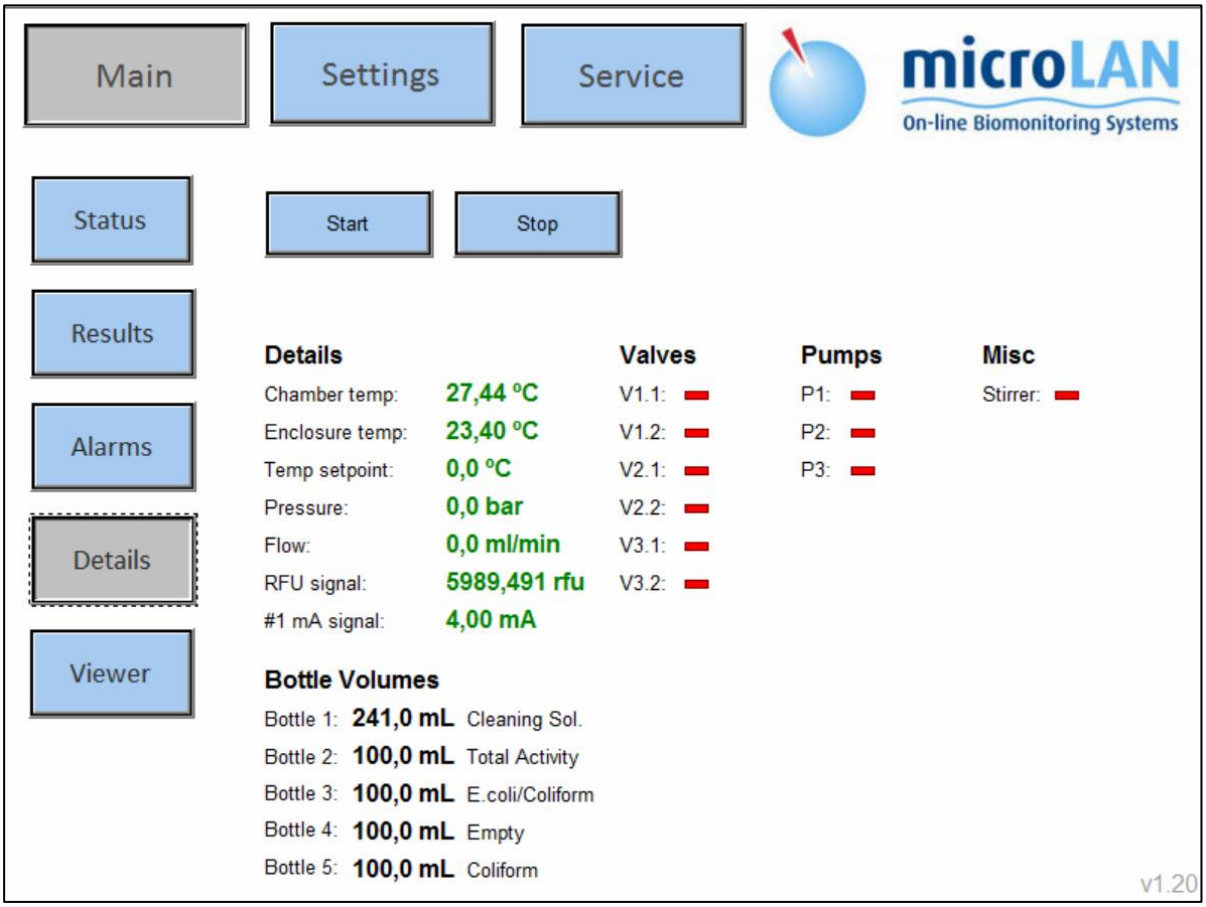


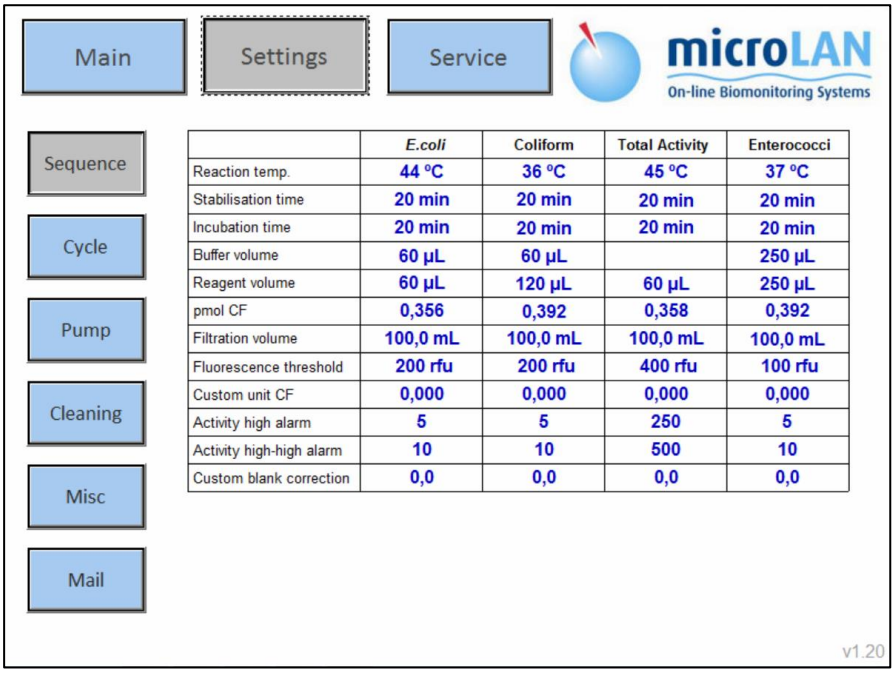
Figure 16 Details screen

7.5 SETTINGS

In the Settings menu process parameters and settings can be changed.

7.5.1 SEQUENCE

In the submenu Sequence, different parameters, such as temperature, stabilisation time, incubation time, conversion factor and filtration volume can be changed.

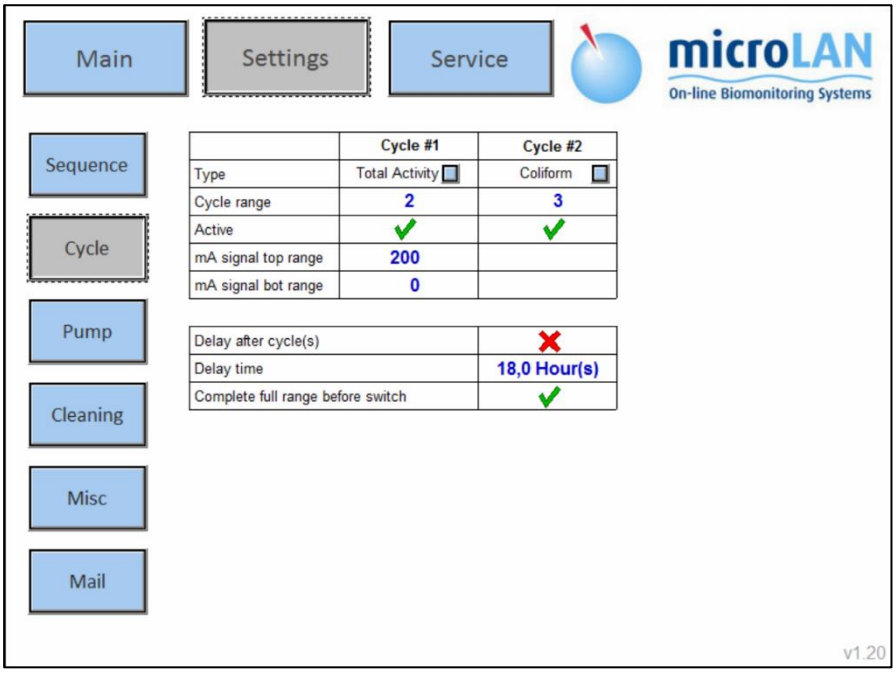


	E.coli	Coliform	Total Activity	Enterococci
Reaction temp.	44 °C	36 °C	45 °C	37 °C
Stabilisation time	20 min	20 min	20 min	20 min
Incubation time	20 min	20 min	20 min	20 min
Buffer volume	60 µL	60 µL		250 µL
Reagent volume	60 µL	120 µL	60 µL	250 µL
pmol CF	0,356	0,392	0,358	0,392
Filtration volume	100,0 mL	100,0 mL	100,0 mL	100,0 mL
Fluorescence threshold	200 rfu	200 rfu	400 rfu	100 rfu
Custom unit CF	0,000	0,000	0,000	0,000
Activity high alarm	5	5	250	5
Activity high-high alarm	10	10	500	10
Custom blank correction	0,0	0,0	0,0	0,0

Figure 17 Sequence submenu screen

7.5.2 CYCLE

In the submenu **Cycle** the settings for the different measurements can be changed. By clicking the blue buttons the measurement can be chosen (E.coli, coliform, Total activity).



	Cycle #1	Cycle #2
Type	Total Activity ☐	Coliform ☐
Cycle range	2	3
Active	✔	✔
mA signal top range	200	
mA signal bot range	0	

Delay after cycle(s)	✘
Delay time	18,0 Hour(s)
Complete full range before switch	✔

Figure 18 Cycle submenu screen

7.5.3 PUMP

In the **Pump** submenu the pump settings can be changed

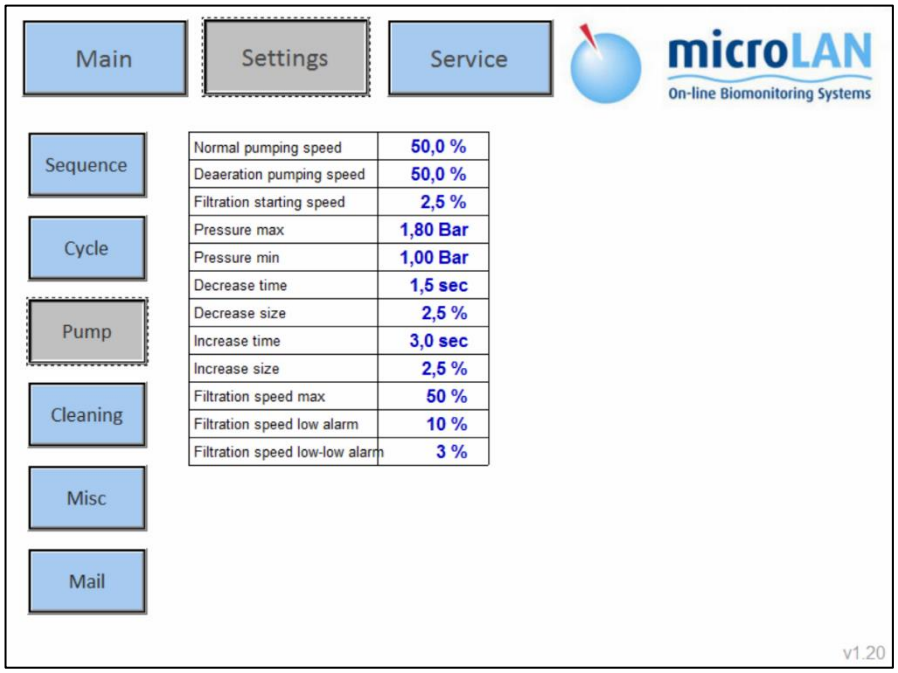


Figure 19 pump screen

7.5.4 CLEANING

In the **Cleaning** submenu the cleaning settings can be changed

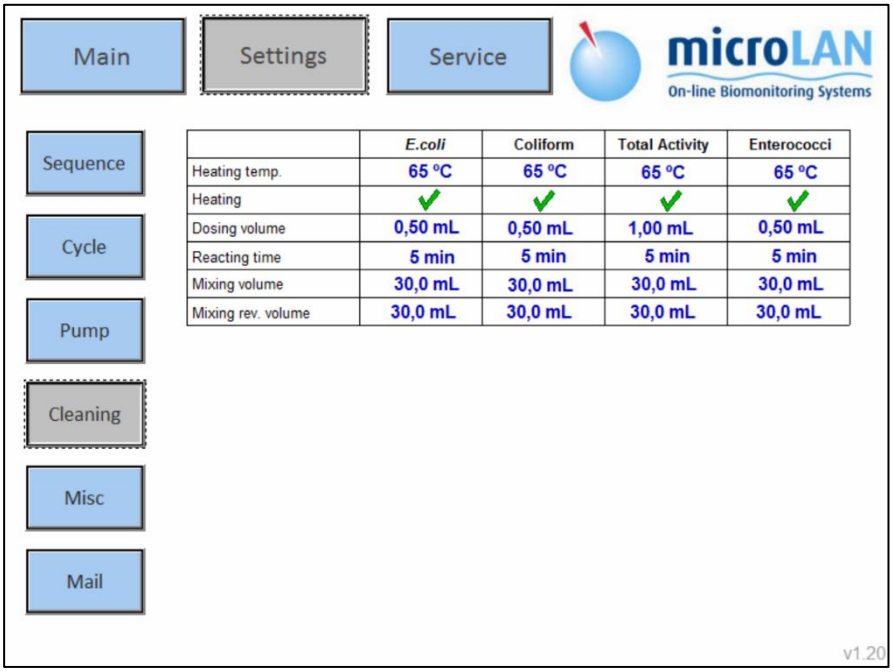


Figure 20 cleaning screen

7.5.5 MISC

In the **Miscellaneous** submenu the limits and delay settings can be changed

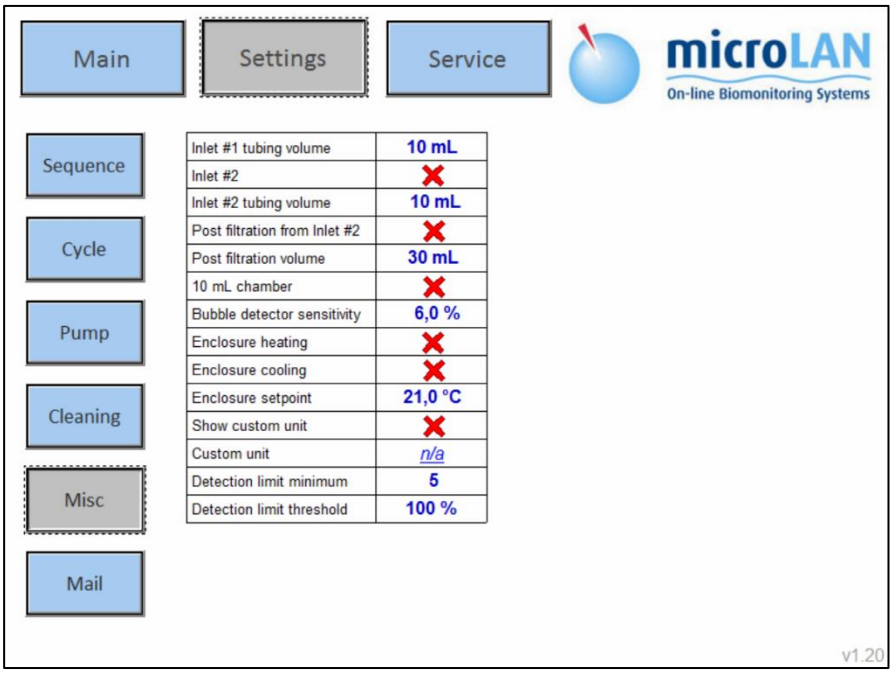
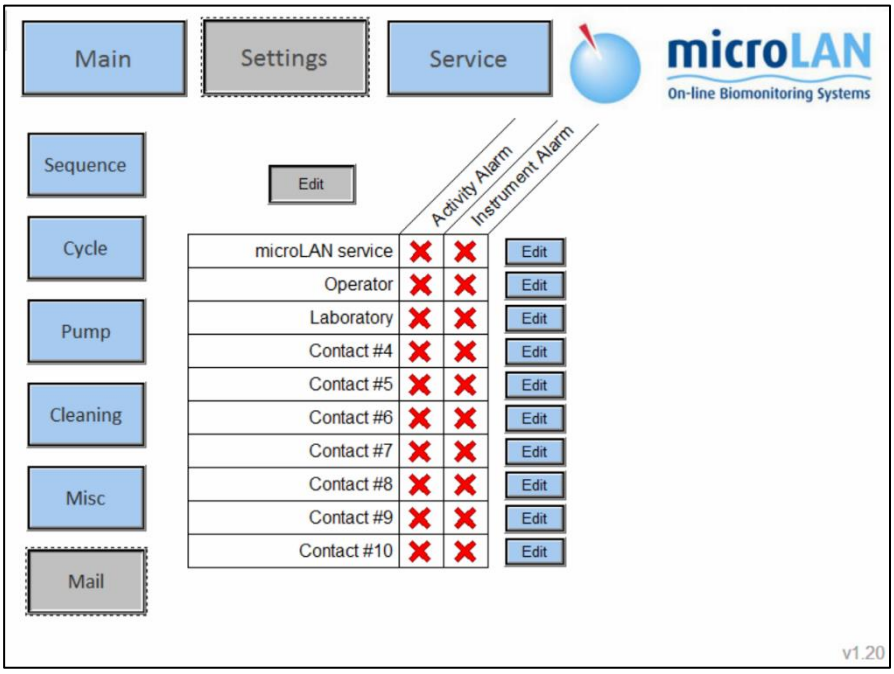


Figure 21 Misc screen

7.5.6 MAIL

In the Mail menu, contact details for automatic generated alarms of the BACTcontrol can be set. Settings for alarms can be appointed to individual users.



7.5.7 CHANGE A VALUE IN A SUBMENU:

- Double click on an item to change.

  Now a pop-up window appears

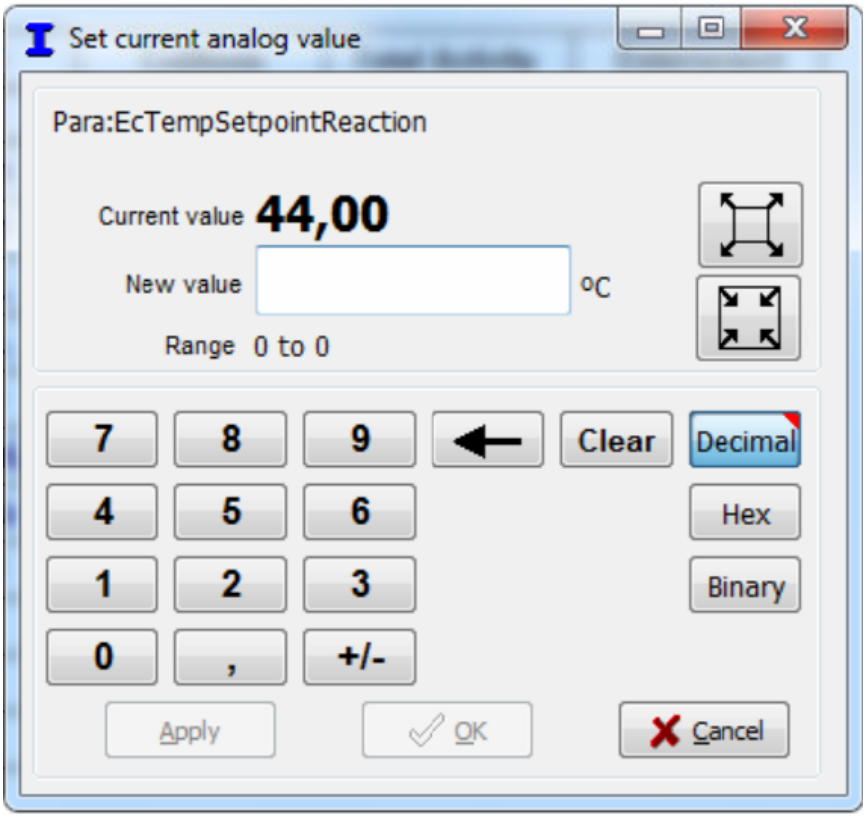


Figure 22 Change value

- Change the value and press **OK** (It is recommended to use Dec.(decimal).

7.6 START A MEASUREMENT

- To start a new measurement click on 

Now a pop-up window appears

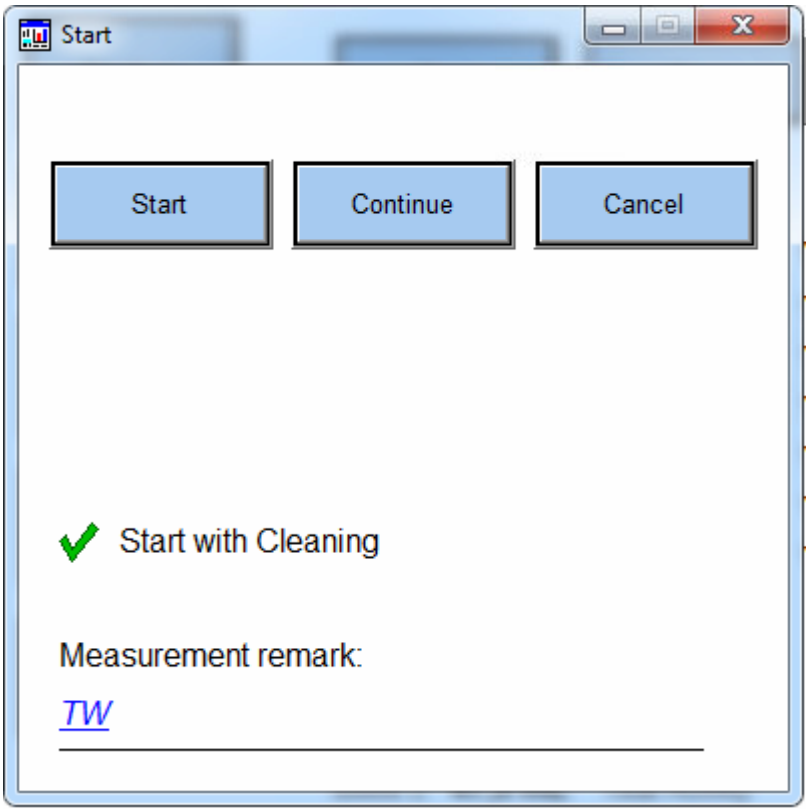
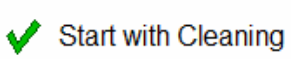
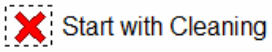


Figure 23 Measurement start menu

There are three options available:

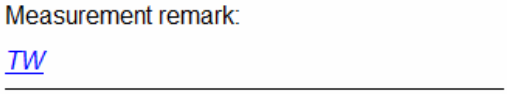
I. **Start** will start a normal measurement. In 

mode the measurement starts with a cleaning first.

To start a measurement without cleaning double click to 

II. When **Continue** is pressed the BACTcontrol immediately will start measuring the next sample, proceedings regarding the preparation of the sample, such as heating, are skipped.

III. Pressing **Cancel** will close the pop-up window.
(no further actions will take place)

Remark:
Before starting a measurement a remark can filled in 

The set points and current status of the measurements are displayed on the main window.

Further details of the last measurement, especially the activity that is measured in the last measurement are displayed under Results

7.7 STOP A MEASUREMENT

- To Stop a measurement press Stop

Now a pop-up window appears

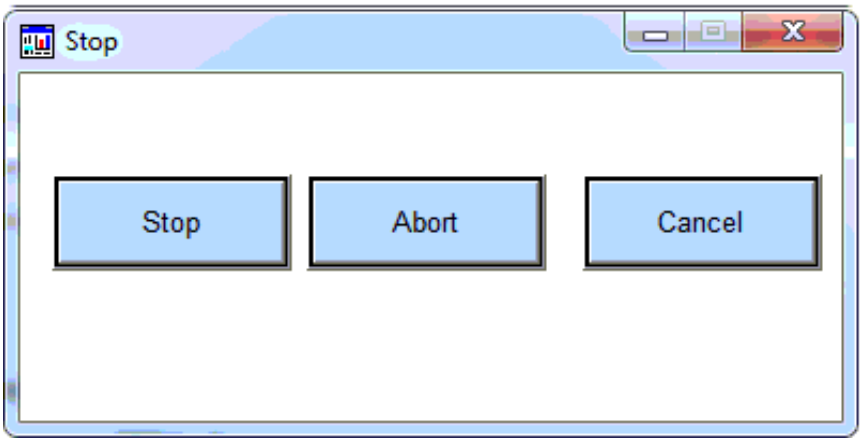


Figure 24 measurement stop menu

There are three options available:

I. **Stop**, the BACTcontrol will finish the current measurement according to the normal procedures;

II. **Abort**, the BACTcontrol will immediately stop with the current measurement, no data or results are saved;

III. **Cancel**, no measurement will be stopped nor aborted. The BACTcontrol will finish the current measurement and will go further with a next measurement.

7.8 SERVICE

In the service menu individual program steps can be activated manually. This can be necessary after service or refilling reagent etc.

(For more detailed information about service: view the BACTcontrol Software manual)

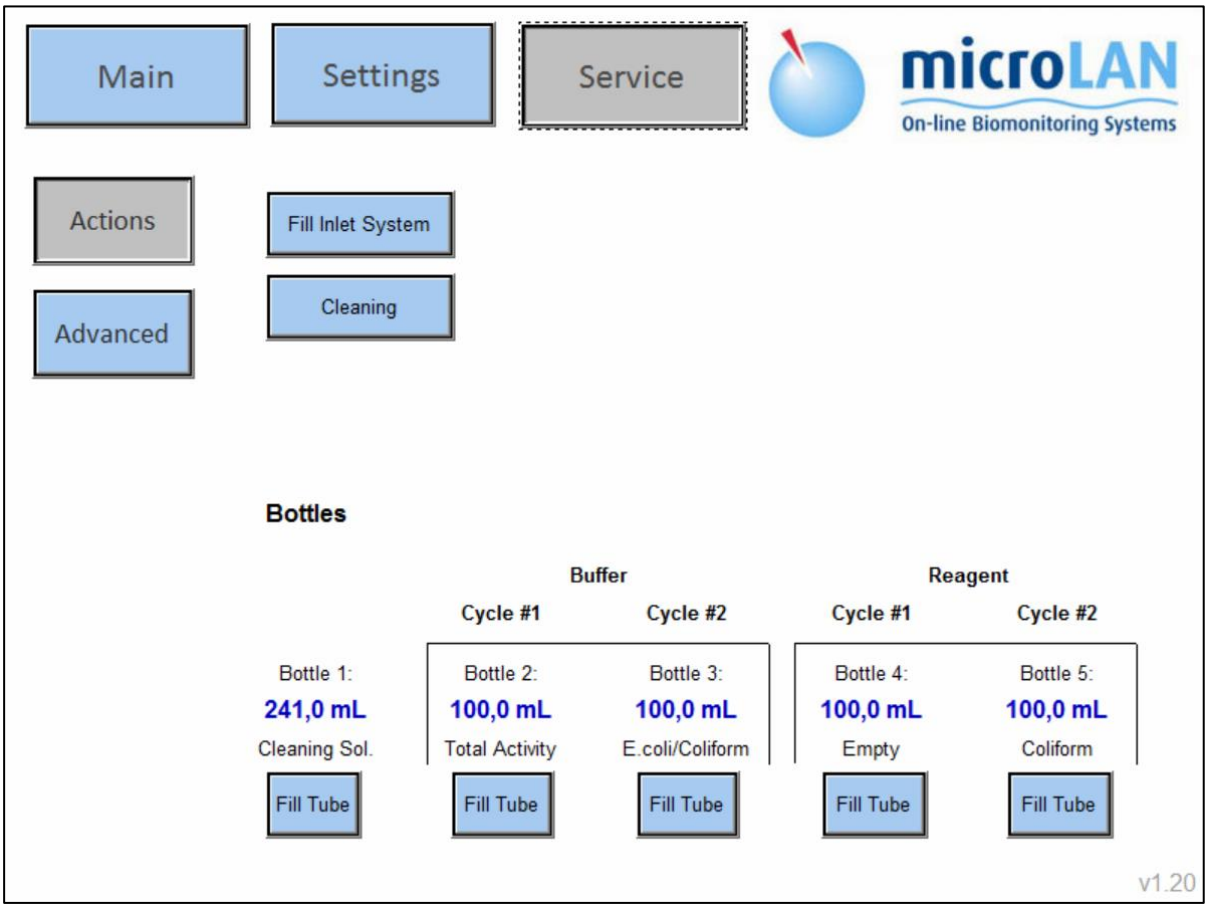


Figure 25 Service menu

7.9 RESULTS

The results of the last measurements can be displayed in a graph

- Go to the status bar on the bottom of the screen
  (only visible when mouse is pointed on it, if not visible, click on 

)

Figure 26 Status bar BACTcontrol

- Press the graph button 

  Now a graph appears

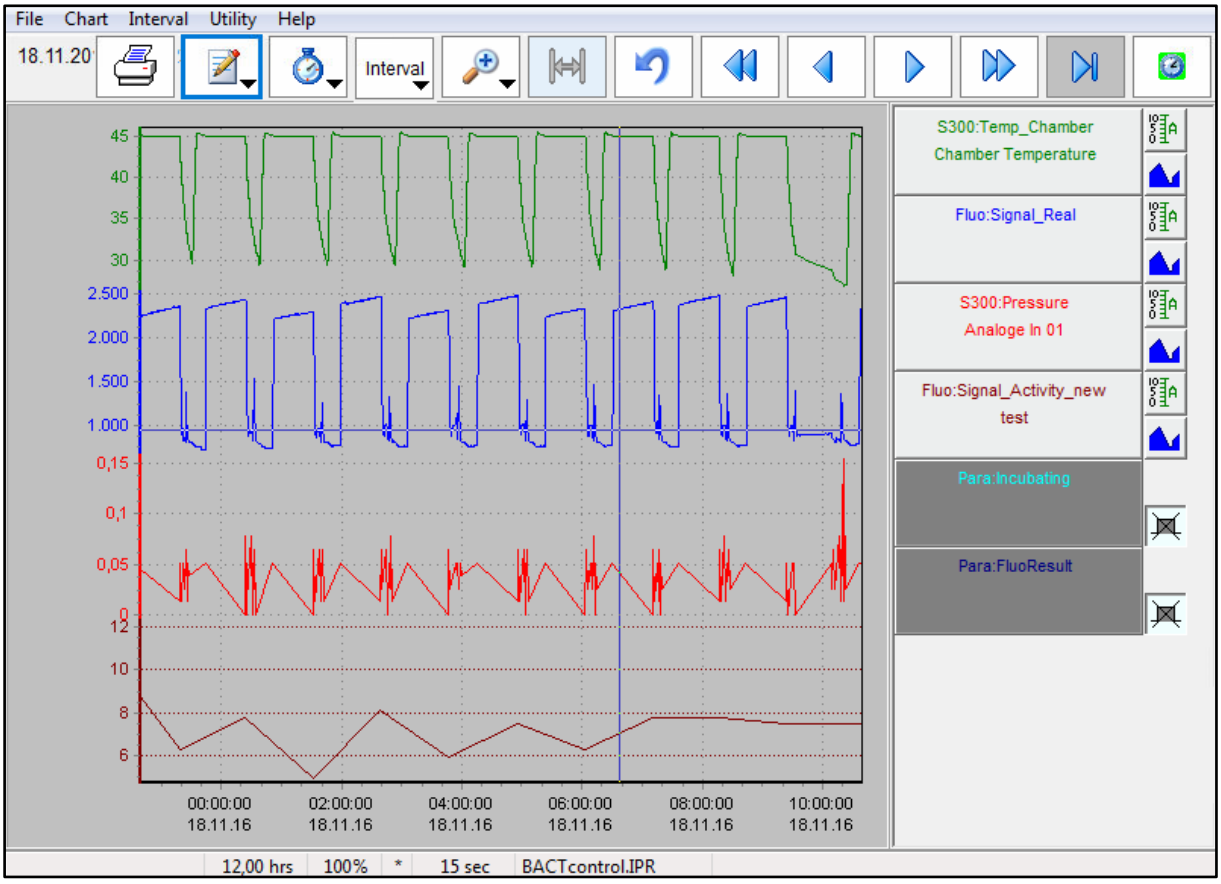


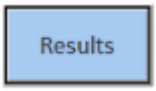
Figure 27 Graph data BACTcontrol

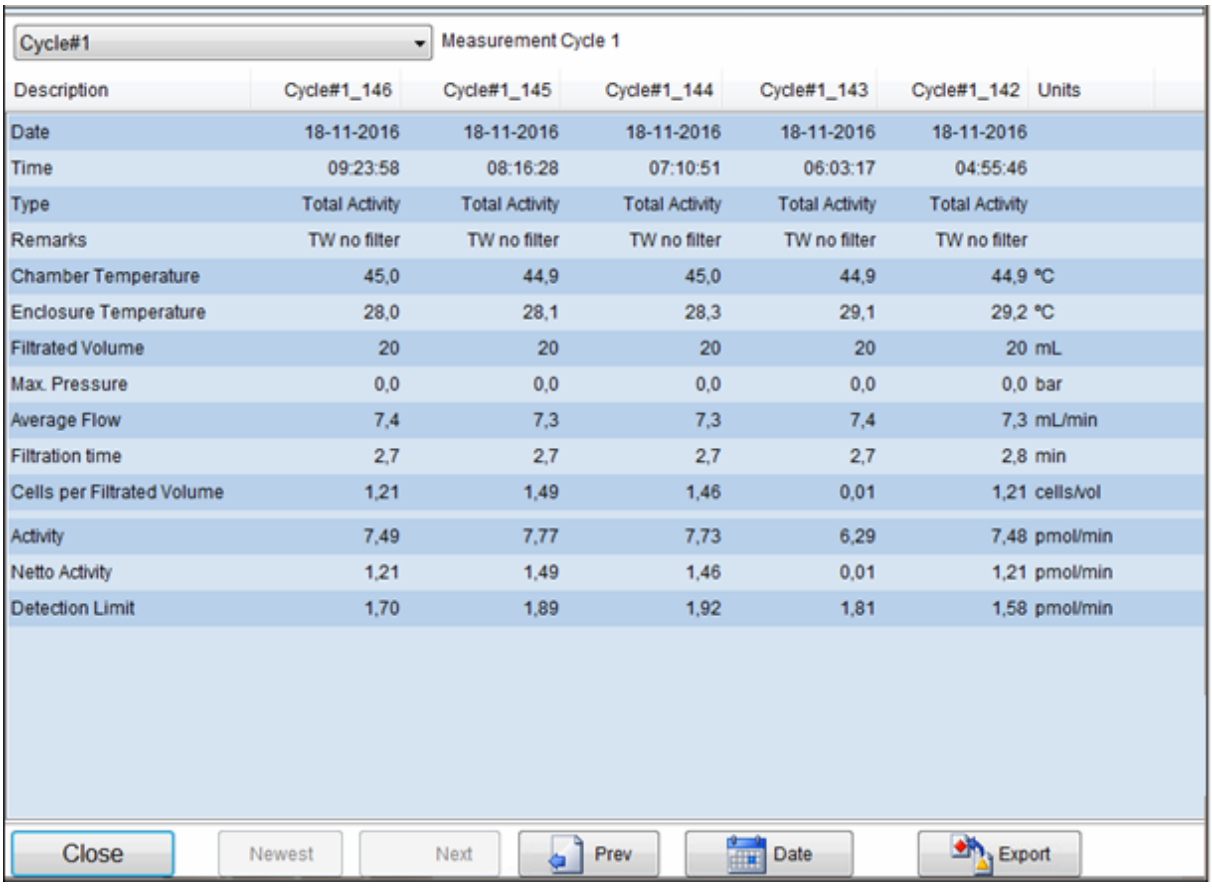
- Move the cursor along the graph to see the results of a specific time on the right side of the window.
- A specific time frame vary from 1 hour to 1 month can be selected with 

- Close the graph by clicking on 

7.10 DATA EXPORT

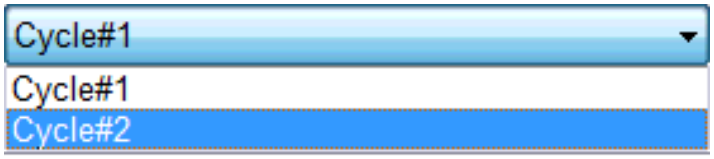
Stored data can be exported to a USB-stick.

- Go to the main display and press 



Description	Cycle#1_146	Cycle#1_145	Cycle#1_144	Cycle#1_143	Cycle#1_142	Units
Date	18-11-2016	18-11-2016	18-11-2016	18-11-2016	18-11-2016	
Time	09:23:58	08:16:28	07:10:51	06:03:17	04:55:46	
Type	Total Activity	Total Activity	Total Activity	Total Activity	Total Activity	
Remarks	TW no filter	TW no filter	TW no filter	TW no filter	TW no filter	
Chamber Temperature	45,0	44,9	45,0	44,9	44,9	°C
Enclosure Temperature	28,0	28,1	28,3	29,1	29,2	°C
Filtrated Volume	20	20	20	20	20	mL
Max. Pressure	0,0	0,0	0,0	0,0	0,0	bar
Average Flow	7,4	7,3	7,3	7,4	7,3	mL/min
Filtration time	2,7	2,7	2,7	2,7	2,8	min
Cells per Filtrated Volume	1,21	1,49	1,46	0,01	1,21	cells/vol
Activity	7,49	7,77	7,73	6,29	7,48	pmol/min
Netto Activity	1,21	1,49	1,46	0,01	1,21	pmol/min
Detection Limit	1,70	1,89	1,92	1,81	1,58	pmol/min

Figure 28 Results screen

- Choose the measurement cycle 

- Press 

Now a pop-up screen will be displayed.

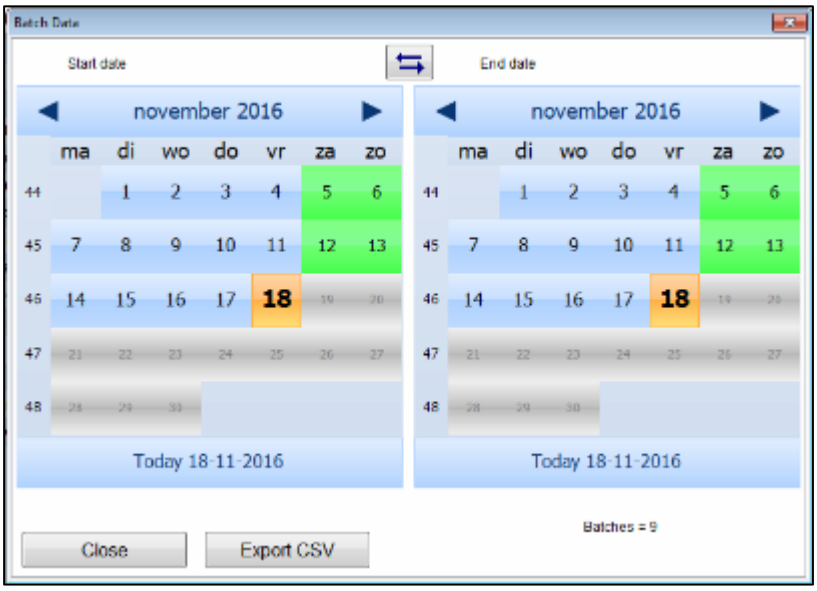


Figure 29 Calendar screen for data export

- Choose the Start date (left) and End date (right)
- Press 

  The data will be stored on:
  Windows > documents > I:\microLAN\IndProj\BACTcontrol\Export.

- Copy the data to an USB-stick (CSV-file can be imported in Excel)

8. MAINTENANCE SCHEDULE

This chapter describes the maintenance schedule.

Always use the original BACTcontrol spare parts which are only provided by microLAN and microLAN distributors.

8.1 DAILY MAINTENANCE

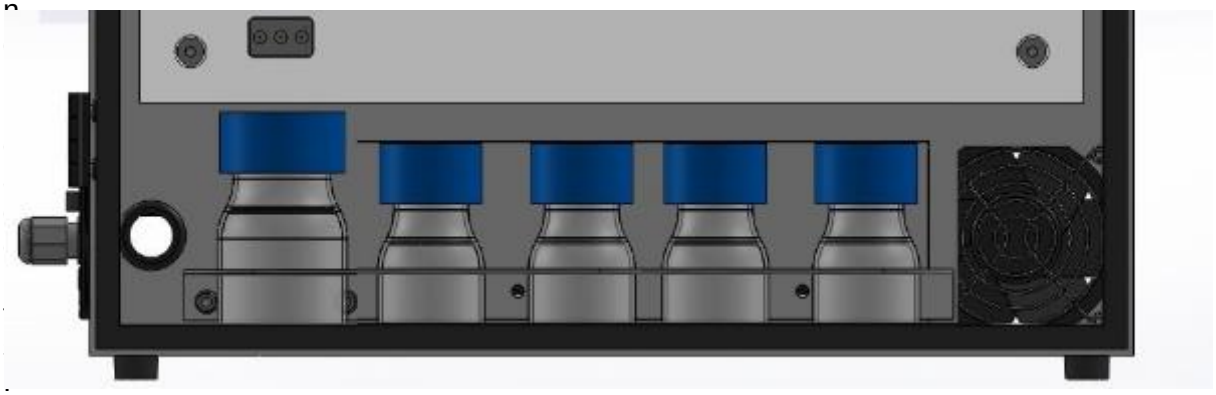
Daily maintenance can be done on any time the instrument is visited. This is not really necessary for the instrument but can be seen as extra attention to the instrument

8.1.1 VISUAL CHECK

- Open the front of the BACTcontrol.
- Check visually if tubes are connected properly and if there are no leakages on the instrument.
- When leakages are detected on the connectors of the tubing, tighten nuts.
  (Be careful ! do not over tighten nuts)

8.1.2 VOLUME SOLUTIONS

- Check the volume of the cleaning solution, buffer a
n



- If necessary place a new bottle. In menu 

press:

- Choose the bottle to replace or fill, and double click on 

its value,

  Now a pop-up window appears

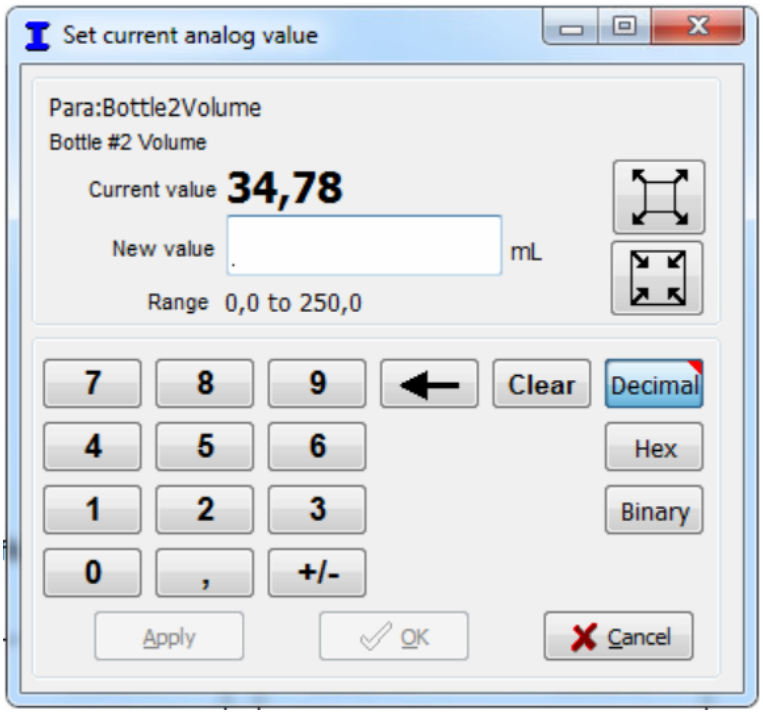


Figure 30 Replace bottle menu

- Change the value and press **OK**

8.1.3 SUPPLY AND WASTE CONTAINERS

- Make sure the sample supply is clean and working properly
- If applicable, check the volume of the waste container and empty if necessary.
- It is recommended to replace the supply tubing on a regular basis (3 to 6 months).

8.1.4 DATA CHECK

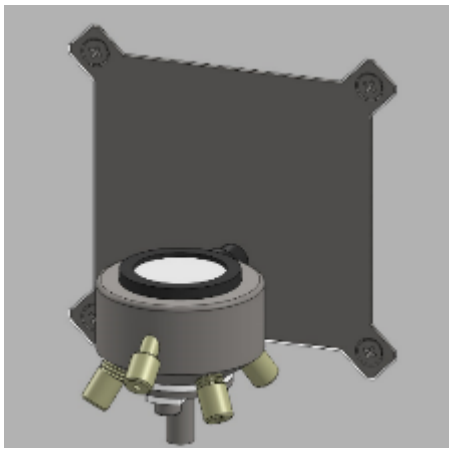
- Check the data and possible alarms on the display of the BACTcontrol

8.2 MONTHLY MAINTENANCE

8.2.1 CLEANING REACTION CHAMBER

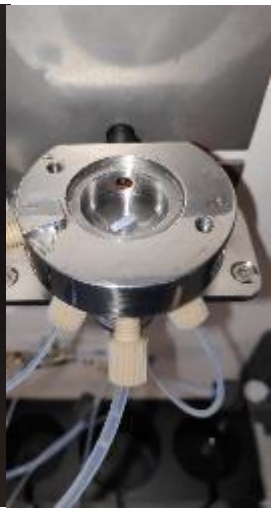
Opening reaction chamber and remove filter

- Remove the cover of the reaction chamber. (unscrew nuts)
- Remove the ceramic filter with the C-ring and check if they are still suitable (not cracked/broken).



Remove stirrer

- Remove the stirrer with the stirrer removal tool
  Be careful! Stirrer is very small



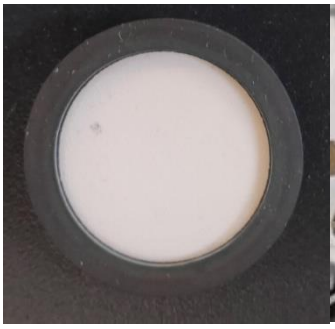
Cleaning reaction chamber and stirrer

- Check for possible biofilm or pollution inside the reaction chamber.
- Clean the measurement cell, the glass of the lens and the stirrer with the cleaning solution and dry with a paper cloth

Reinstall the stirrer and filter and closing reaction chamber

- Place the stirrer in the reaction chamber.
- Place the ceramic filter with the C-ring, make sure that it is installed with the upside on top.

The upside is marked with a small dot as shown in the figure on the right. Close reaction chamber and turn the knob hand-tight.
Make sure that cover is placed properly to prevent any leakage.



8.3 QUARTERLY MAINTENANCE

(required interval depends on type of sample water)

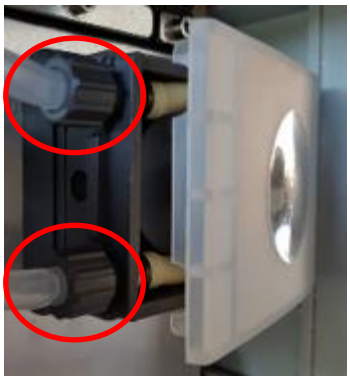
8.3.1 REPLACE TUBING

(to prevent pollution or scaling on the tubing it is recommended to replace the tubing every 3 to 6 months)

To remove the tubing from the sample inlet, Second inlet, waste outlet and sample outlet

- First press the metal one touch fitting, then pull the tubing out of it.
- To place a new tubing, just insert the tubing in the one touch fitting.

8.3.2 PERISTALTIC PUMP TUBE CHANGING



- Switch off the device.
- Remove the external tubes connected to the peristaltic unscrewing fixations

- Remove pump cover and pump rotating cover

- Remove the peristaltic pump tube subset part.
- Re install a new one and do all actions in reverse order. Re connect the external tubes
- Switch on the device again.

- Check the liquid aspiration.

8.4 PREVENTIVE MAINTENANCE (YEARLY)

Preventive maintenance can be performed by your distributor.

During preventive maintenance the instrument will be completely checked and tested.

If Available, software updates will be performed.

For more information about this preventive maintenance contact your local distributor.

9. REAGENTS, CLEANING SOLUTION AND BUFFERS

Table 4 reagents, solutions and buffers

	Reagents
02BACT001A262ml50	E.coli activity reagent 50 ml (for reaction chamber 2 ml)
02BACT001A262ml100	E.coli activity reagent 100 ml (for reaction chamber 10 ml)
02BACT001A263ml50	Coliform activity reagent 50 ml (for reaction chamber 2 ml)
02BACT001A263ml100	Coliform activity reagent 100 ml (for reaction chamber 10 ml)
02BACT001A264ml50	Buffer reagent 50 ml (for reaction chamber 2 ml)
02BACT001A264ml100	Buffer reagent 100 ml (for reaction chamber 10 ml)
02BACT001A265	Cleaning stock solution 100 ml
02BACT001A268ml50	Total activity reagent 50 ml (for reaction chamber 2 ml)
02BACT001A268ml100	Total activity reagent 100 ml (for reaction chamber 10 ml)
02BACT001A413	Cleaning agent for hard water H2O2 / CH3CO3H 1.000 ml
02BACT001A426ml50	Enterococci Substrate 50 ml (for reaction chamber 2 ml)
02BACT001A426ml100	Enterococci Substrate 100 ml (for reaction chamber 10 ml)
02BACT001A427ml50	Enterococci Buffer 50 ml (for reaction chamber 2 ml)
02BACT001A427ml100	Enterococci Buffer 100 ml (for reaction chamber 10 ml)

MSDS are available on request by microLAN and microLAN distributors

10. Consumables

Table 5 Consumables

	Consumables
04BACT001A254	BACTcontrol filter 24mm Ø set (3 units with filter holder) for reaction chamber 2ml
04BACT001A255	BACTcontrol filter placeholder open 24mm Ø for reaction chamber 2ml
04BACT001A256	BACTcontrol filter placeholder closed 24mm Ø for reaction chamber 2ml
04BACT001A429	BACTcontrol Filter 47mm Ø set (3 units with filter holder) For reaction chamber 10ml
04BACT001A702	BACTcontrol filter placeholder open 47mm Ø for reaction chamber 1
04BACT001A703	BACTcontrol filter placeholder closed 47mm Ø for reaction chamber
04BACT001A247	Sample inlet (FEP tubing black) 2M
04BACT001A251	Output waste,4mm Ø, 2M
04BACT001A252	Syringe 2cc 5 pc
04BACT001A490	Tubing with couplings complete set vs1 (to sn 00-0020)
04BACT001A560	Tubing with couplings complete set vs2 (starting from sn 00-0021)
04BACT001A518	Peristaltic tube replacement

11. SPARE PARTS

Table 6 Spare parts

	Parts
06BACT001A026	Reactor temp.sensor (sn 00-0001 - 01-0020)
06BACT001A027	Reactor heating element (sn 00-0001 - 01-0020)
06BACT001A048	Fluorescence sensor
06BACT001A053	Pressure sensor
06BACT001A054	3-Way valve
06BACT001A055	Micro dosing pump
06BACT001A075	Liquid sensor (sn 00-0001 - 01-0020)
06BACT001A081	Axial fan
06BACT001A118-A	Control board S300
06BACT001A121	Fanless PC
06BACT001A242	Take up rod for magnetic stirring bar
06BACT001A253	Stirrer bar white, 2x7mm, 2 pack
06BACT001A258	Cooling unit (only for bottles)
06BACT001A516	Enclosure Cooling unit
06BACT001A269	Cartridge fuse 2A 5 pack
06BACT001A272	Cartridge fuse 1A 5 pack
06BACT001A273	Voltage regulator (sn 00-0001 - 01-0020)
06BACT001A278	Main pump (sn 00-0001 - 01-0020)
06BACT001A304	Optical Mouse
06BACT001A340-B	Stirrer motor mx01 (sn 00-0001 - 01-0020)
06BACT001A383	Enclosure temp.sensor
06BACT001A392	Enclosure heater
06BACT001A539	Reactor heating element (starting from sn 00-0021)
06BACT001A540	Reactor temp.sensor (starting from sn 00-0021)
06BACT001A545	Stirring Motor Assy (starting from sn 00-0021)
06BACT001A555	Peristaltic Pump (starting from sn 00-0021)

12. SOFTWARE

Table 7 Software

	Software
07BACT001A257	Software license (sticklock)
07BACT001A491	Software sticklock USB
07BACT001A522	USB-Nano-RS485 converter

REFERENCES

1. Lopez-Roldan, R., Tusell, P., Cortina, J. L., & Courtois, S. (2013). On-line bacteriological detection in water. *TrAC Trends in Analytical Chemistry*, *44*, 46-57.

2. Munson, L., & Fall, R. (1978). Purification and characterisation of Escherichia coli alkaline phosphatase. A biochemical experiment. *Biochemical Education*,*6*(3), 53-56.

3. Ryzinska-Paier, G., Lendenfeld, T., Correa, K., Stadler, P., Blaschke, A. P., Mach, R. L., & Farnleitner, A. H. (2014). A sensitive and robust method for automated on-line monitoring of enzymatic activities in water and water resources. *Water Science & Technology*, *69*(6), 1349-1358.

4. Sigma-Aldrich (-) 4-methylumbelliferone http://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/aldrich/product_information_sheet/m1381pis.pdf . Consulted on 30-12-2015

APPENDIX A: GENERAL WORKING OF THE BACTCONTROL

The concentration of E.coli, coliforms and total activity are determined based on the presence of enzymes which are specific for these bacteria. Each enzyme has its own optimum so the measurements must be done separately, meaning that during each sequence only one type of enzyme can be detected.
The sample goes through the inlet into the reaction chamber, where is concentrated in the ceramic filter and is brought into the temperature, depending on the enzyme to be determined. For a general view of the BACTcontrol flow scheme see Figure 31.

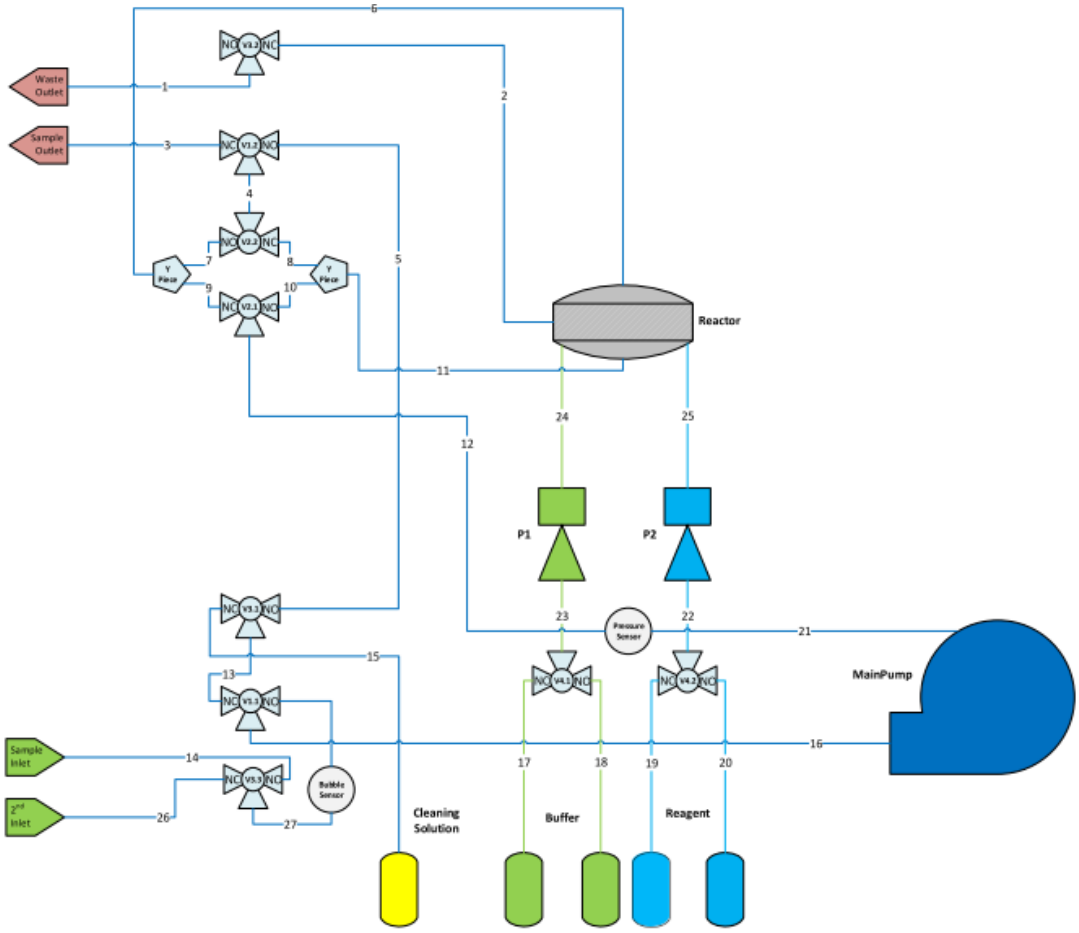


Figure 31 Schematic flow of the BACTcontrol

The volume to be filtered has to be set by the user, which might vary with the type of water. The volume to be pumped can be set, with a minimum of 100 up to a maximum of 1000 millilitres.

When the sample is at the correct temperature the reagents are pumped into the reaction chamber and the incubation time starts. During the incubation the photodiode measures the fluorescence produced of enzymatic activity which increases when the bacteria are present in the sample. The measured fluorescence is converted into amount bacteria per 100 millilitres of measured sample.

Figure 32 shows the general measurement process of the BACTcontrol. It describes the different steps involved in the measurement including parameters like time, volume, temperature and dosing of reagents.

Initiation	Filtration	Measurement	Cleaning
System filling	0,2 – 1 ml/s	Temp. 36-45 C. 96.8 - 113 F.	Temp. 65-70 C. 149 -158 F.
Rinsing & cleaning	Volume depending	Heating of rector	Heating of reactor
All pumping operations through the filter in reverse direction	Filter condition depending	Buffer & reagent dosing & stabilisation. Stepwise measurement of the fluorescent signal	Disinfection of system with chlorine solution

Figure 32 General measurements steps

When the measurement begins, after reaching the adequate temperature (Coliform 36 °C / 96,8°F, E.coli 44 °C / 111,2°F) and adding the buffer solution and reagents, the bacterial enzymatic activity starts. The enzymes start a metabolic process, converting large substrates into smaller substrates or products. The reagents added to the reactor contain the substrates needed for the measurements. Due to the fluorescent characteristic of these substrates it is possible to measure the enzymatic activity and therefore an approximation of the bacterial population can be made. The fluorescence is determined during the “Measurement” steps. For a detailed explanation of the working of the enzymes see the next paragraph.

ENZYMES

Enzymes are large proteins structures responsible for thousands of metabolic processes. Enzymes can either launch a reaction or speed it up (catalyst). In enzymatic reactions, substrates are converted into different molecules, called products (Figure 33). Almost all chemical reactions in a biological cells, including bacteria like E.coli and coliforms, need enzymes. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathway occur in that cell.

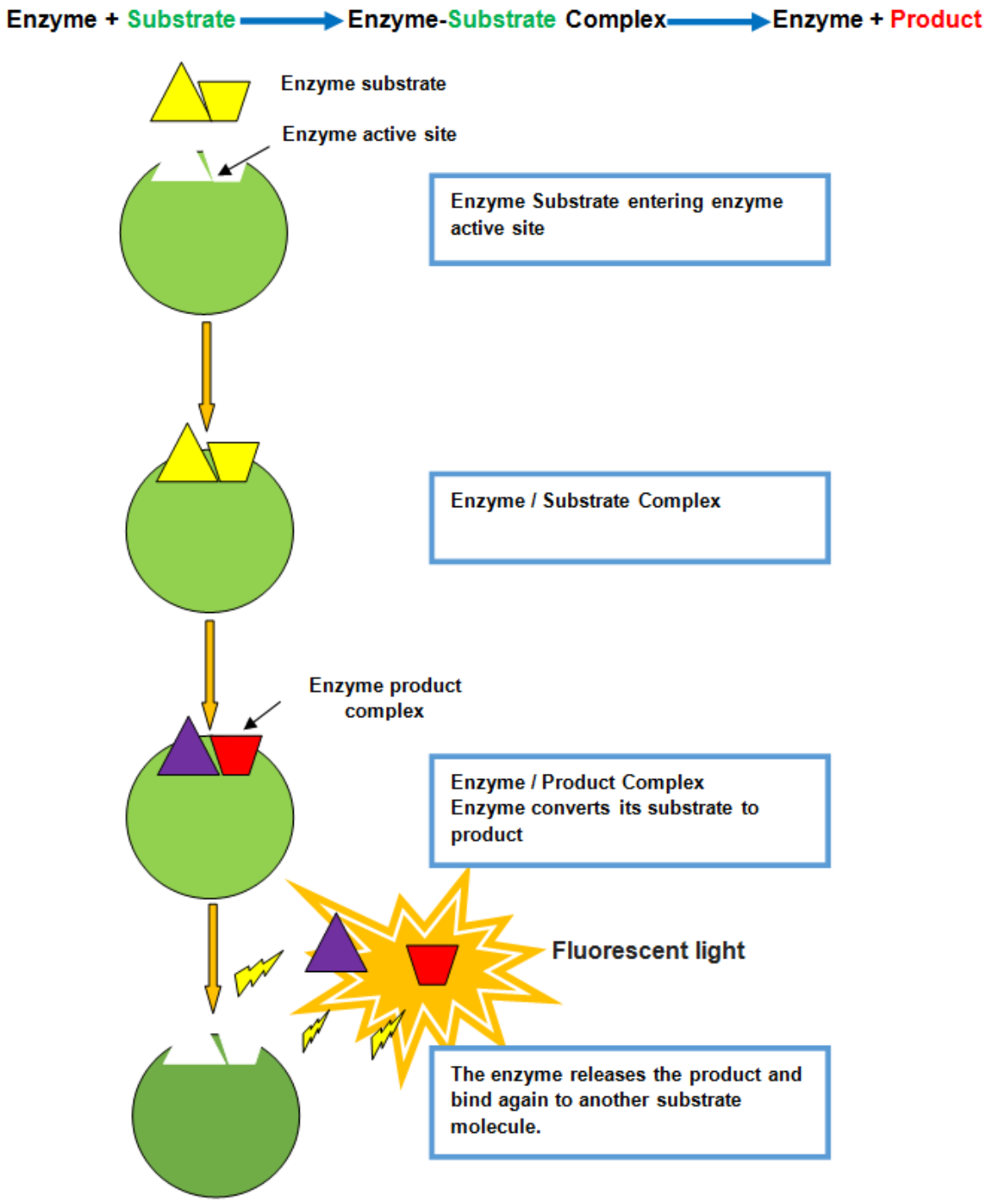


Figure 33 General working of enzymes

In the reaction chamber the enzyme comes in contact with the substrate. As shown in the figure 33 above, the enzymes attach to the substrate previous to the conversion, meaning that the enzyme can convert one molecule substrate at the time. Enzymatic activity is expressed as the amount fluorescent substrate that is produced (REF. 3). When a substrate molecule is converted, the enzyme goes further with the next substrate molecule. In the first stage of the measurement, the signal will increase gradually therefore a stabilization period is necessary. When the signal is stable, then the conversion of measured fluorescence to specific enzymatic activity takes place. In the BACTcontrol, enzymatic activity is expressed in picomole per minute.

When the molar mass of an enzyme and the amount of converted substrate are known, then the activity of the enzyme can be expressed as the molecular activity in mol per time unit. One picomol equals 10^{-12} mol.

Alkaline phosphatase is an enzyme that catalyzes the hydrolysis[1] of a chemical structure. Like the name says, it is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. This is called dephosphorylating process. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. The optimal activity is at pH 8,0 at 25ºC / 77°F, at pH 10 at 37°C / 98,6°F.

The purpose of the enzyme is not completely known. The hypothesis is that it is a means for the bacteria to generate free phosphate groups for uptake and use. This is supported by the fact that alkaline phosphatase is usually produced by the bacteria only when the concentrations of phosphate in the environment are low.

For this reason this technique is useful as warning system on the production of drink water, where the concentrations of phosphate are low. Beta-galactosidase is an enzyme that catalyzes the hydrolysis of beta-galactosides into monosaccharide (short molecules of sugar). In coliforms, the gene of β-galactosidase, is present as part of an inducible system which is activated in the presence of lactose when glucose level is low. This inducible system is activated by the addition of the reagent that corresponds to coliform bacteria.

Beta-Glucuronidase is an enzyme that catalyzes the hydrolysis of complex carbohydrates. Beta-Glucuronidase from *E. coli* is capable of very quickly hydrolyzing of many compounds. β-Glucuronidase is used for the enzymatic hydrolysis of glucuronides in biological fluids, primarily urine. This enzyme is activated by the addition of the E.coli reagent.

The hydrolysis product (MU) fluoresce when it comes into contact with the proper enzymes. The grade of activity of the enzyme catalyzing the hydrolysis is determined by an increase in fluorescence per time. The fluorescence can be detected under long wave UV light (366nm) as a blue or green fluorescence.

[1] Hydrolysis, hydro, meaning water, and lysis, meaning separation usually means the cleavage of chemical bonds by the addition of water.

APPENDIX B: CHECKLIST

Checklist			
BACTcontrol	Maintenance Schedule Checklist	Form: mL8025	microLAN On-line Biomonitoring Systems

Rev.: 0.4 / CK
Date: 16-sep-19

Year /		Monthly/Quarterly		Yearly	

		Item:				
		By:				
		Date:				
		Check general condition BACTcontrol	yes / no			
		Visual check on leakage	yes / no			
		Check sample supply and waste storage	yes / no			
		Visual check volumes solutions	yes / no			
		Check Negative control (chlorinated water)	yes / no			
		Clean measurement cell and glass lens	yes / no			
		Check pumps and valves	yes / no			
		Clean tubing	yes /no			
		Replace tubing	yes / no			
		Replace pump tubing	yes / no			

Other service:

Notes: