Operation Manual

BacTrac 4300

Part 1: Device Manual

Part 2: Microbiological Manual

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Part 1: Device Manual

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1 General

1.1 General warnings

The BacTrac 4300 system was not designed for medical diagnostic applications and must not be used for such.
The device may only be operated by qualified laboratory personnel. Users need sufficient microbiological expertise to interpret results correctly and to make decisions based on them. SY-LAB is in no way liable for any damage (direct or indirect, material or immaterial) that may occur in connection with the use of the device or the software by the user or third parties.

1.2 Short Description / Intended Use

Rapid Microbiological Analyser
The new Bacteria Tracer 4300 can automatically and rapidly detect contamination and bacterial growth of a great variety of microorganisms.
In the recent past, SY-LAB has become the market leader for impedance analysers because of the sturdy construction and versatility of our instruments.
Due to its innovative measuring system, a vast amount of applications becomes possible.
Two impedance values per analysis, the M- and E-Value offer more flexibility for selection of a variety of different types of growth media as well as enhanced security for sterility testing.

BacTrac 4300 combines a high sensitivity for detection of metabolic product changes with the possibility to use a big range of highly selective growth media.

Short pay-off and economic analysis
Simplicity of sample preparation as well as reduction of preparation steps due to automatization decreases costs and time per analysis. Preparation setups such as handling of plates, preparation of dilution steps or counting and evaluation of results are eliminated.

The consistent and readily available documentation increases the informative value and saves the operator valuable time. Other aspects such as shorter stock turn-over and increase in quality control have to be considered as well. The applications of an impedance analyser such as the BacTrac 4300 are nearly endless.

BacTrac analyser systems can consist of up to 12 individual instruments operating at two different temperature levels each controlled by one PC.

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1.3 Warranty

SY-LAB Instruments GmbH will grant a warranty of **12 months** starting on the date of delivery, for all products manufactured by SY-LAB, with the exception of those components so noted in the warranty. It is guaranteed that every instrument is free of material or manufacturing defects which can cause alterations in the appropriate measurements intended to be performed with the BacTrac. Our obligations within the constraints of this warranty include repair, service, and necessary instrument adjustment where, after expressed, written permission from SY-LAB is given, products are sent to our service facility, and here the instrument defects can be confirmed. SY-LAB will pay the shipping costs of such, only under the condition that the shipment of defective parts follows the written agreement of SY-LAB and the shipment conditions stated by SY-LAB.
SY-LAB will assume no liability for damage whether directly or indirectly resulting from defective functioning of the instrument, software, or material defects if such liability is not stated in the appropriate product liability code.

Note: The warranty conditions stated above do not apply to re-sellers.

Should an instrument or product be judged defective within the time of the warranty, it is the discretion of SY-LAB to supply parts which may be installed by the customer, for example, printed circuit boards, temperature sensors, etc.

Warranty claims are not valid in the following cases:

a) Damage from improper use,
b) Forceful use,
c) Improper installation by persons non-representative of SY-LAB,
d) Unauthorized repair or alteration of instrument parts,
e) Materials for consumption (measuring cells, nutrient media, etc.) or
f) Damage from environmental influences.

The materials used in SY-LAB products were selected after much experience and intensive testing of reliability and stability.

Exceptions to the 12-month warranty:

1) **Spare parts**
Spare parts are guaranteed for a period of **90 days** after the date of delivery. This applies to defects in the material or in the construction of the spare parts. SY-LAB is not responsible for such defects when any persons not authorized in writing by SY-LAB perform repairs or instrument modifications.
2) **Consumable Materials**
Articles of consumption such as test tubes, O-rings, fuses, nutrient media, etc. are exempted from this guarantee, with the exception of any manufacturing defects. Parts which function normally for 45 days after the date of delivery will be considered free from manufacturing defects.

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2 Soft- and Hardware-Specifications

2.1 BacTrac 4300 Technical Description

Microbiological Analyzer – detection by impedance-splitting technology

Dry-Incubator

Each BacTrac 4300 unit contains two dry-block incubators with 32 sample wells each, that are independently temperature controlled. Up to 12 units (768 wells, 24 incubators) can be controlled by one PC. Each sample well has high-reliability electronical contacts that accommodate either disposable or reusable measurement cells. Each incubator section is covered by a convenient, easily cleaned glass cover.

Temperature Control

The temperature range of each 32 well incubator section can be adjusted in software by the control PC from 0 °C to 60 °C and maintain with an accuracy of ± 0.1 °C. In addition to temperature information being available on the control PC, each BacTrac 4300 unit has a 2 line vacuum fluorescent display that provides cell location and temperature information. For incubator temperatures near room temperature or below, an optional circulation cooler is required.

Measuring Cells

Direct impedance measurements for bacteria can be performed using either 20 ml disposable plastic or 10 ml reusable glass cells. Glass cells and their electrode assemblies are autoclavable. Disposable plastic cells are sterile and either empty of pre-filled with nutrient broth.. Indirect impedance measurements of yeast and molds can be made using specific cells: a 20 ml reusable outer cell with integral electrodes is used with either a disposable empty (6.5 ml) or disposable pre-filled (7 ml) inner vial that mates with it.

Electronics

Each BacTrac 4300 has a built-in computer subsystem for incubator temperature control, impedance measurements, fault monitoring and bi-directional communication with the control PC. Impedance measurements are made at 1 KHz and each sample cell location is polled every 10 minutes.
Incubator set points are controlled by the PC and maintained by the 4300's on board computer.

Computer specification - minimum requirement

Only a PC that demonstrably meets the requirements of the EN 60950-1 or EN 62368-1 standards may be connected to the device.

Intel Pentium IV or comparable processor, 2.0 GHz, 1 GB RAM, VGA, hard disk >10 GB, CD-ROM, 1 x RS-232 per BacTrac 4300, 1 x USB per BacTrac 4310, 1 x USB for local printer (not necessary if network printer is available), monitor 17 "(with 1-6 incubators, 19" recommended with 7-12 incubators), screen resolution 1024 x 768 pixels, color printer, operating system Microsoft® Windows ™ 7, 8 (. 1) or 10 (Pro / Professional).

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Software

MS Windows, menu-driven, automatic time-date registration, continuous sample monitoring, color-coded detection time thresholds and evaluation status indication, rapid identification of analysis status.
Allocation (including for individual positions) of analysis parameters and calibration curves, easy text input and editing, flexible indication and sorting parameters, report generation, LAN connection, data export opportunities, data interfaces to external programs available.

Dimensions, weight

Dimensions: 430 mm (W) x 640 mm (D) x 380 mm (H) (16.9 x 25.2 x 15.0 inches)

Weight: 34 kg (75 lbs.)

2.2 Normal Operating Conditions

Nominal power:	3.9 A {230VAC} or 7.8 A {115VAC} (must be specified at the time of ordering)
Fluctuations of supply voltage:	+/- 10 % max.
Frequency:	50/60 Hz ± 2 %
Ambient Temperature:	+15 to +35 °C
Relative humidity:	15-80 % (non-condensing)
Protection class through housing:	IP 30
Installation location:	Inside buildings
Degree of pollution of the Intended environment:	2 accoring to EN 61010-1
Operating Position:	Level stable surface
Altitude:	0 to 2000 m above sea level
Operation of cooling:	inlet pressure max. 400 kPa (4 bar)

Dimensions, weight and power requirements are for the BacTrac 4300 unit only and do not include the control unit (PC).

2.3 Transport and Storage Conditions

There should be no abnormal behavior under following conditions. However, after long periods of storage, a temperature calibration might have to be performed.

Temperature:	+5 to +40 °C
Humidity:	10 to 90 % relative humidity (non-condensing)

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2.4 Additional Specifications

Protection against data loss in case of power failure
Internal lithium batteries protect the configuration parameters from being lost during a power failure. The life-span of such Lithium batteries is about 10 years under normal environmental conditions.

2.5 Safety Rules and Symbols

Description of symbols used in this handbook:

General Warning

A warning indicates conditions or actions that might lead to damage of the product or severe human injury.

In cases where the general warning symbol is attached, the manual must be consulted to determine the nature of the potential hazard and the resulting actions.

Manufacturer - Indicates name and address of the manufacturer

Date of production

SN **Serial number** of instrument

CE **CE - Marking** of instrument

AC **Alternating current**

Not to be put in household waste

Read instructions!

OFF (Supply)

ON (Supply)

Protective conductor terminal

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2.6 Electromagnetic Compatibility

This instrument complies to safety class I – test voltage 1kV (with protective ground).

This instrument was manufactured according to the following current norms regarding electromagnetic compatibility (EMC) for companies in industrial areas.

EN 61326-1:2013-01

Upon request, EMC test reports are available.

2.7 Periodic Inspections

Safety-related tests according to ÖVE/ÖNORM-EN 62353 or ÖVE/ÖNORM E 8701-1 or IEC 62353 or corresponding Norms have to be performed periodically. The test interval must not exceed 3 years.
The tests have to be performed by skilled personnel. For further information on this, please contact SY-LAB Austria or its representative.

2.8 General Safety Precautions

During operation, maintenance or any other repair work, the following special safety instructions have to be followed.
If these safety instructions and the warnings in this manual are not observed, this constitutes a violation of the safety standards underlying the design, manufacture and intended use of the device and can endanger the user.

SY-LAB will not be responsible for any damage or harm violating the statement above.

- All instruments used concurrently with the BacTrac have to be operated according to the applicable norms. All device connection lines must be connected and screwed if necessary before the mains voltage is switched on.
- Pay attention to all notes, safety and warning messages mentioned in this manual and labels displayed on the instrument.
- If the device is not operated in the manner intended by the manufacturer, the protection provided by the device may be impaired.
- Always disconnect the instrument from the power line before attempting to open, clean or perform any service work. Do not use abrasives or cleaning sprays except when it is asked for in the manual. Use only a wet soft cloth for cleaning unless stated otherwise.
- To change the location of the device, the device must be moved by two people. They have to lift the device with both hands at the same time on the top bar of the side cladding, carry it to the desired location and put it down again.
- The surface on which the device is standing should be sufficiently stable, as the device could be seriously damaged by percussions caused by a fall.
- The metal blocks of incubators A and B can normally get up to 63 °C and in the event of a fault up to 75 °C. 
- The openings on the instrument housing are mainly used for cooling purposes. Do not block these openings, otherwise the instrument might get damaged due to overheating.

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Also, do not place the instrument close to heat sources or ventilation systems such as radiators, fans, etc.

- Before connecting the instrument to the mains, make sure that the voltage rating shown on the type-plate agrees with your main supply. If in doubt, contact your SY-LAB GmbH. representative or SY-LAB GmbH. Austria directly.
- Use only the original power supply cable. A grounded (ground potential) wall socket is required.
- The Instrument must be connected to a wall socket with functional ground potential.
- The electrical requirements of the Room were the Instrument is used has to comply with the national standard. (in Austria - ÖVE-EN 1 or ÖVE / ÖNORM E8001)
- Place any wires coming from instrument such that it is no hindrance to the operator.
- When using extension wires, be sure not to exceed the maximum power rating of all instruments connected.
- Never try to insert objects into any instrument openings. Electrical shock might be the result which in turn might be harmful to life. Do not pour any liquid into any of the openings.
- The system must be set up so that no parts of the computer can come into contact with liquids in the event of a leak in the cooling system.
- Be careful when handling measuring cells containing liquid. Avoid spilling the liquid on system components.
- With the exception of work asked for in this manual, never try to repair the instrument by yourself. Repair work must be performed only by authorized personnel.
- Only accessories provided by the manufacturer may be used.
- With the exception of the procedures expressly stated in the manual, you should never attempt to repair or maintain the device yourself. All maintenance work may only be carried out by authorized specialist personnel. By removing parts of the device, contact with high voltages cannot be ruled out. We therefore emphasize that our devices must be disconnected from the voltage source before any service intervention. **Warning: Danger to life.**

In all following cases, disconnect the instrument from the mains and call your service representative:

- Power supply cable or connector worn or defective.
- Liquid was spilled and entered the instrument.
- Instrument shows malfunction when used according to the operator manual.
  Note: Use only accessories (as mentioned in the operator manual) intended to be used with this type of instrument.
- Instrument was dropped and/or housing is damaged.
- Instrument shows strange behavior as compared to normal operation.
- When instrument was stored for a long period of time.

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2.9 Description of Parts

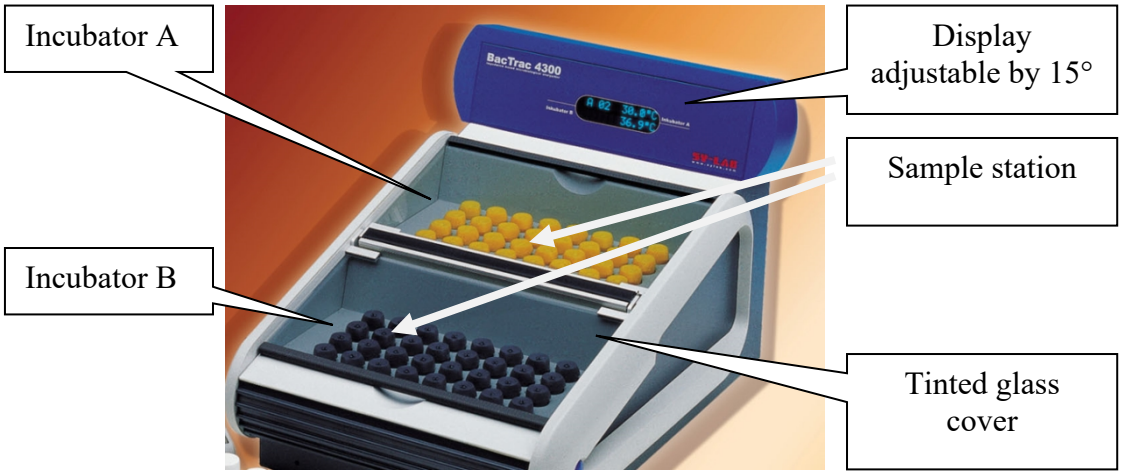

Fig. 1 Front View

2.10 Site Preparation

The following points must be taken into account when selecting the installation site:

- Place the device on a flat, horizontal and stable surface.
- The device must be freely accessible for operation and maintenance work.
- Select the location so that the device is protected from direct sunlight, excessive heat or cold, moisture or dust.
- Only connect the device via a separate socket and do not use an adapter plug (T-distributor).
- A minimum distance of 10 cm must be kept to walls and other objects. Never set up the device in such a way that it is difficult to operate the disconnection device (power plug or device connector or power switch). Do not use sockets that are controlled by wall switches or automatic timers. Accidental power interruption can cause data in the computer and device memory to be lost.
- Do not use any sockets whose circuit also includes large motors or other devices that can cause sporadic supply deficits.
- The computer system and its connected components / devices should under no circumstances be set up in the vicinity of potential sources of interference (e.g. loudspeakers or base units of a radio telephone).
- Devices, computers and other system components must be connected to the same power supply (same potential). Differences in potential caused by poor earthing of one or more sockets can damage the devices.

2.11 Environment

- The instrument will work without any difficulties under normal working conditions (15–35 °C / 60–95 °F). Nevertheless, in a very humid environment, excessive condensation can lead to the destruction of the instrument. The environment should be such that 80 %

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humidity (non-condensing) is not exceeded. Also, during transportation, it is vital that the instrument is given time to adjust to the surrounding temperatures before turned on, especially in the wintertime, when instruments are transferred from a cold to a warm environment or vice versa.

- Instruments on a trolley must be secured against movement. Use the roller-lock on the wheels to do so.

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3 Installation

Caution: The safety of a system into which the device is integrated is the responsibility of the system installer.

3.1 Unpacking

Fig. 2 Shipping box

The instrument will be shipped in a cardboard box. The outer card board hood is fixed with four white plastic clamps. Take them out and lift the hood.
Caution: Do not try to lift out the BacTrac by yourself since the BacTrac is heavy. Remove the box with accessory materials. Carefully remove the styrofoam panels and place the instrument on a flat, dry and clean stable surface.

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3.2 Contents Check

After unpacking, inspect if all parts are complete and undamaged.

Note 1: Missing parts damaged parts must be claimed immediately.

Part Description	Part No.	BA	EX	
Power cable	00-155540	1		for 220 VAC instr.
PC connecting cable, 1,4 m Type USB A-B	40-431694	1		
PC connecting cable, 4,5 m Type USB A-B	40-431695	1		
Power distribution box for 5 conn.	40-415115	1		Not for 110 VAC instr.
Power distribution box for intern. power cable	40-415116	1		
Sample rack 16 Pos.	40-431700	4		
Temperature sample cell	40-431760		x	
Cooler connection kit	40-431730		x	For 2 Instr. in series *
Cleaning box	40-419899		x	
Rack f. indirect cells, 21 pos.	40-414735		x	
Cooler connection kit with valve block	40-419950		x	For 3 Instr. in parallel
Disposable and re-usable sample cells	various		x	
Power cable	00-155538	1		For 110VAC instr.

Note 2: For operation, a personal computer as well as the operation software 40-499300+ is necessary (see the technical specification for PC minimum requirements)

BA= Basic accessory,
EX= Extra accessory (needs to ordered separately)
* = not recommended for temperature differences of more than 5 °C between incubators.

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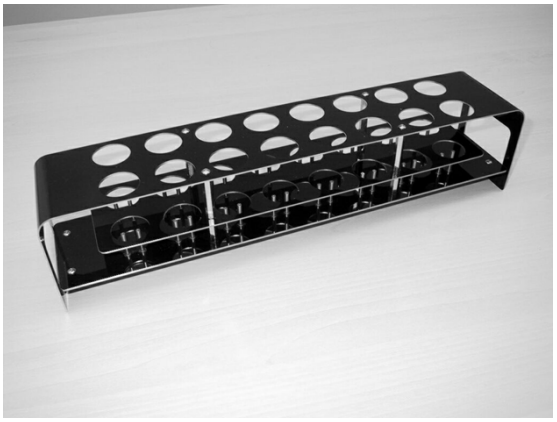

Fig. 3 Sample rack

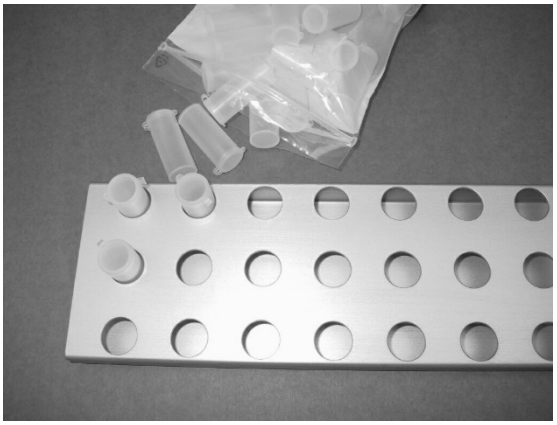

Fig. 4 Indirect sample cells and rack

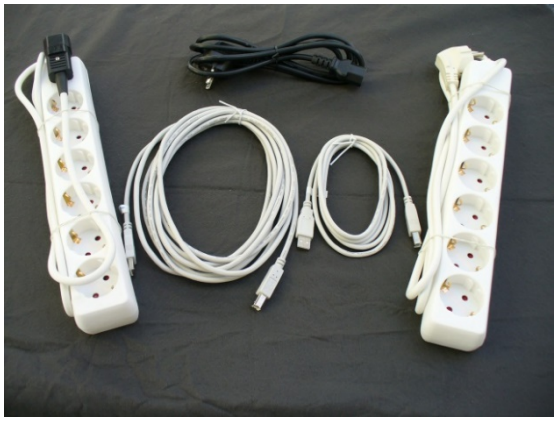

Fig. 5 Power and PC cables

Do not dispose of the instrument's packing material. It is part of the instrument and should be kept for possible later transportation.

Caution: Check the voltage rating of your instrument. The voltage mentioned on the instrument's type-plate must match the local supply voltage. If not, do not connect the instrument to the power line. Immediately contact your local supplier or SY-LAB Austria directly. The supply voltage was set at the factory and cannot be changed by the end-user.

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3.3 Connections

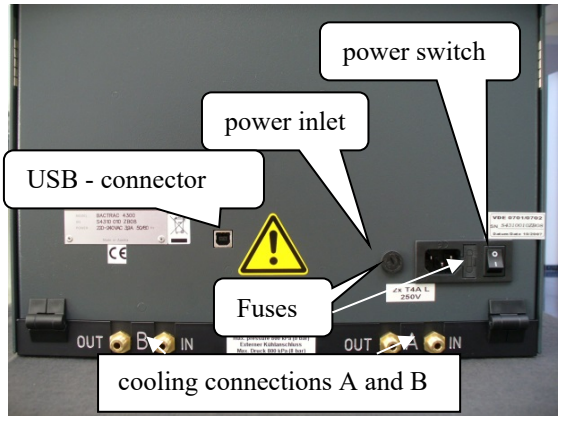

Fig. 6 Back view

The power input is located on the back side of the instrument. Use only the power cable supplied with the instrument. Next to the power inlet, you will find the main switch as well as the fuse compartment.
If other than the original power cord are used (regional standards), they must offer the same level of protection as the original cable. Two-pole cables without a ground connection must not be used. In addition, the length must not exceed that of the original cable and the cross-section must not be less than that of the original cable. The power cord used must be certified.
When operating the instrument at 220 V AC, use only 250 V fuses according to IEC-Norm 127 (5x20 mm).

Caution: Before connecting the instrument to the power line, verify the proper line voltage (see instruments type plate)

Electrical connection – personal computer (PC) or laptop
The BacTrac will be operated by software and requires a proper computer to function correctly (see specification).

USB communication connection
A USB type A-B cable is needed to connect the BacTrac to the PC. The setup of the correct port will be set in the operating program of the PC. Use only the original communication cable.

Fig. 7 USB port

Cooler connection
On the backside of the BacTrac, there are two sets of connectors for hooking up a cooler. The temperature of each block segment can be adjusted independently and can vary as much as 15 °C to each other.

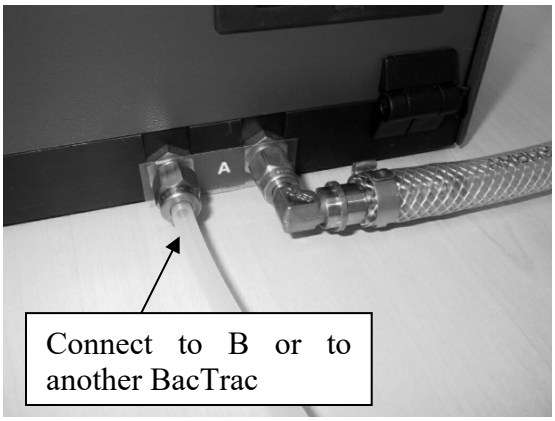

Fig. 8 Water connector A

The operating temperature of the BacTrac can vary between 0 °C and 60 °C. A cooler is also required if the operating temperature is close (around 7 °C) to environmental temperature or below.
The connection pressure may not exceed 4 bar. Suitable coolants are ultrapure water (ion exchanger) or distilled water to which 0.1 g / l sodium carbonate (Na_2CO_3) has been added to reduce the corrosive properties. Other

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coolants must not be used. The temperature of the coolant must be between 4 and 50 ° C.
The flow direction is not important.
Normally, only a slight counter-cooling is required. In cases where the cooling medium is too cold, the heating element might not be able to handle the temperature compensation and thus, the expected temperature cannot be reached.

Cooler connecting kit (optional)
For each BacTrac, a Cooler Connection Kit is available. It consists of the following parts:

- Tap with short hose and bend
- Cooler connection hose
- clamps
- bend M10x1
- wrench, 7 u. 8 mm
- wrench, 12 u. 14 mm

(see Fig. 9)

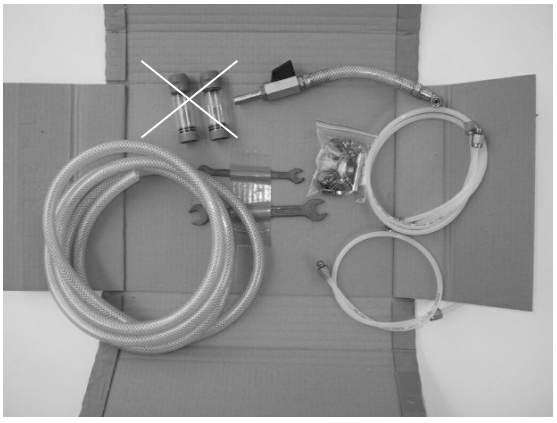

Fig. 9 Cooling connection kit

If external cooling is required, connect the kit as shown below.

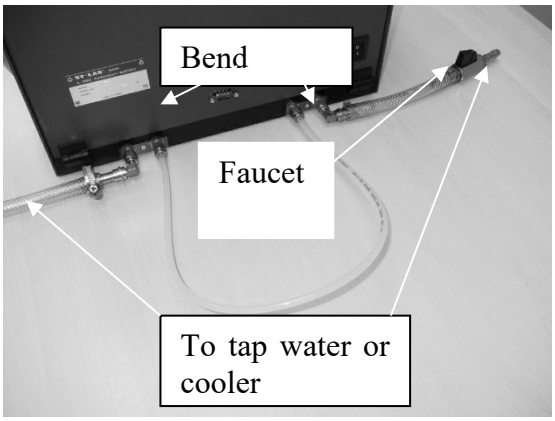

Fig. 10 Tubing connection

3.4 Fuses

The fuse holders are located next to the power inlet. Use a small screw driver to pull the fuse holder out. 220V AC instruments require two (5 x 20 mm) Slow Blow 4A/250V fuse. The power cable must be removed before the fuse can be changed.

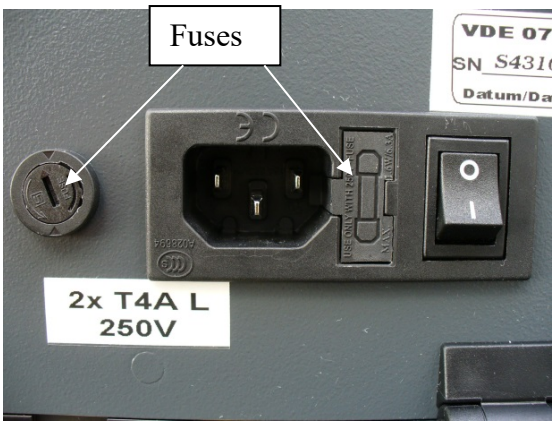

Fig. 11 Power inlet / fuse holder

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4 Maintenance and Service

Maintenance and service work may only be carried out by SY-LAB authorized and trained persons. All parts required for this must be obtained from SY-LAB or an authorized sales partner.

The contact sockets, which are installed on the bottom of each measuring station of the device, are wearing parts that have to be replaced depending on the intensity and duration of use. It is recommended to have their condition checked by the manufacturer or sales partner during an annual inspection.

4.1 Calibrating the BacTrac 4300 Temperature Sensors

If it is found, that the temperatures within the BacTrac measuring positions are not within the specified limits (set temperature ±0.5 °C)

Material needed:

- Calibrated thermometer
- Filled measuring cell (e.g. water)

We suggest the following procedure:

Turn the BacTrac on.
Start the *BacMonitor* program.
Fill a measuring cell with water and place it into position 29.
Insert the sensor of your calibrated thermometer in the measuring cell and close the cover as good as possible.
Wait until the measuring cell reaches equilibrium. SY-LAB recommends waiting at least for one hour after the turn on just to be sure that temperature equilibrium has been reached within the incubator block and that the measuring cell remains at a constant temperature.

For precise calibration, we recommend to use an electronic thermometer. An adapter should be used to position the temperature sensor into the center of the filled measuring cell. An overnight measurement would be preferable.

Now enter the Menu *Extras / Temperature Calibration* and enter the password *SyTmpCal*. A window like the one in Fig. 12 will be displayed:

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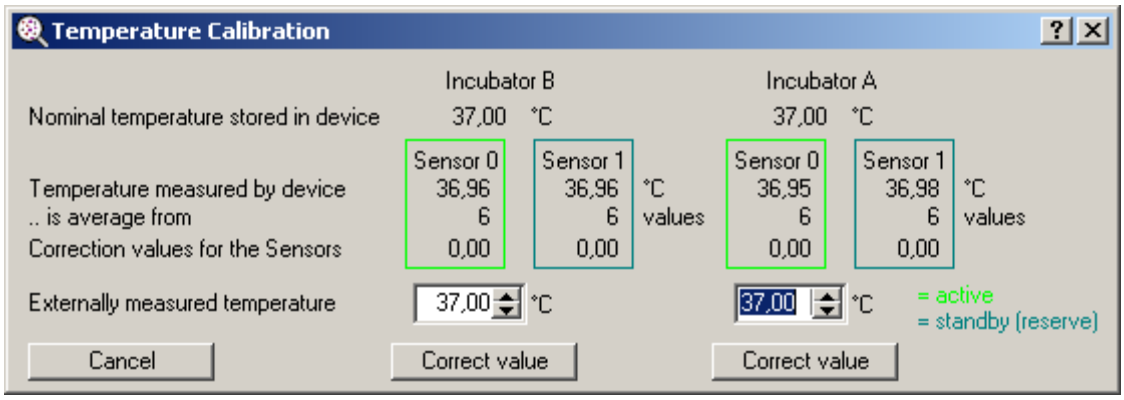

Fig. 12 Program window for temperature calibration

Each incubator block of the BacTrac 4300 has two temperature sensors. One active (main) sensor is used for controlling the temperature in the block. The other sensor is a spare and will be activated only in case the main sensor fails.

For the calibration, it is necessary to calculate the average of at least six temperature measurements. If there are less than six temperature values available, the field *Correct value* will be high-lighted.
Now insert into the field *externally measured temperature* the value as measured by your thermometer and acknowledge with *Correct value*.
The fields "*is average from"* will be reset to zero and the correction values of the sensors will be newly determined. Subsequently, the field *Correct value* will be high-lighted again (as long as there are at least another six temperature readings)

Note:
The value of the spare temperature sensor will be displayed only during measuring OFF-intervals and it might be necessary to wait until the end of the measuring cycle in order to get the necessary 6 temperature readings.
It is not necessary that the BacTrac reaches the externally determined temperature entered previously. However, it is necessary that the temperature calibration is done only when the incubator blocks have reached a steady temperature (about one hour after turn-on).
For the calibration, choose the temperature where most of your measurements will be done.

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5 Disinfection and Cleaning

5.1 Disinfection

In the event of contamination of the device or individual measuring stations, e.g. if the contents of the measuring cells are spilled, the device should be disinfected. To do this, most of the contaminated liquid should first be removed with an absorbent cloth. The contact base cleaner (Fig. 5.2) can be used in the measuring stations. The surface is then sprayed with 70% ethanol. After an exposure time of 2 minutes, residues of the disinfectant are removed with an absorbent cloth. Disinfected measuring stations must be completely dry before further measuring cells can be used there.

5.2 Description of cleaning set

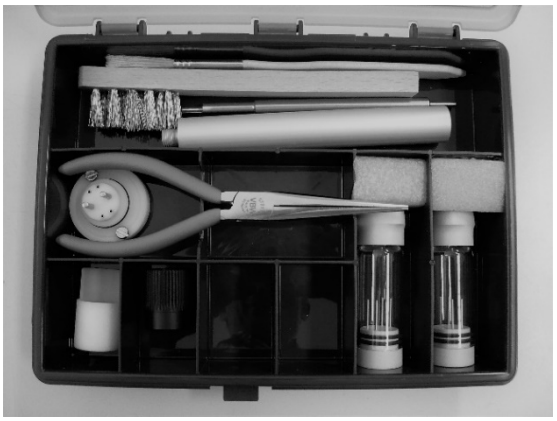

Fig. 13 Cleaning set

Fig. 13 shows a complete set of cleaning tools. Use only the tools that are recommended by SY-LAB. Other tools might damage your instrument.

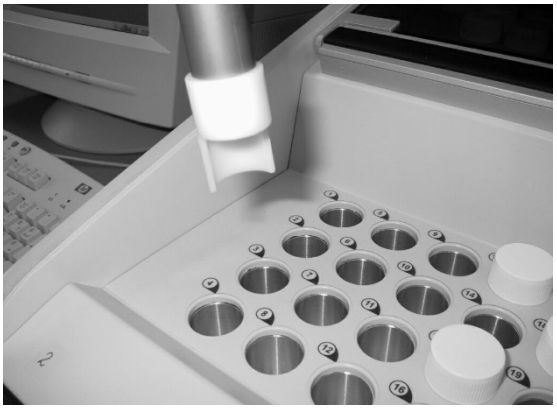

Fig. 14 Contact socket cleaner

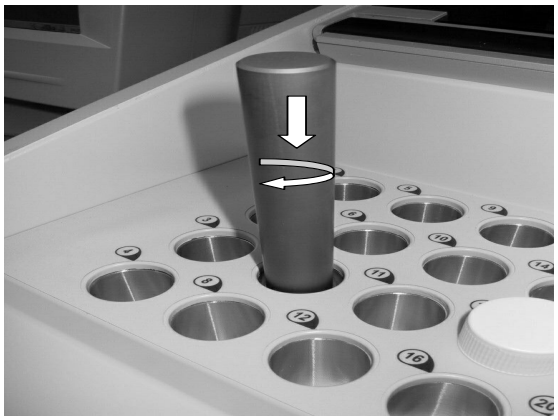

Fig. 15 Extender tool

The tool in Fig. 14 and Fig. 15 is used to clean the contact socket at the bottom of the incubator positions. In case of liquid spills, use paper towels or a cloth wrapped around the contact cleaner to absorb the liquid

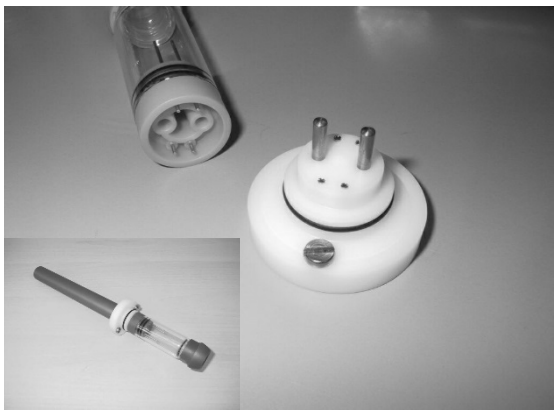

Fig. 16 Contact socket

Fig. 16 shows a contact socket. It is used as a holder for assembling or disassembling measurement cells. The insert shows the tool

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with the measuring cell. When a measuring cell is dismantled, always replace the O-rings.

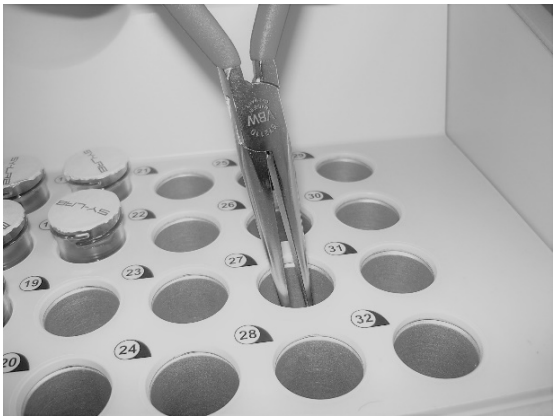

Fig. 17 Pliers

The pliers in Fig. 17 is useful to pull out any contact sockets from within the incubator position in case the measuring cell came apart.

Fig. 18 Guiding rod scraper

The tool in Fig. 18 is used for removing stains and encrustations from within the contact socket.

Fig. 19 Milling head for meas. position

The milling tool in Fig. 19 is used for scrapping off any dirt on the walls and bottom of the measuring position.

Fig. 20 Scraper for contacts (1)

Fig. 20 and Fig. 21 show how the contacts of a contact socket is cleaned using the contact scrapper.

Fig. 21 Scraper for contacts (2)

In addition, the kit also includes a cleaning brush, wire brush and two spare sample cells. The wire brush is used for cleaning the milling tool.

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6 Appendix

6.1 Accessories

Only the accessories provided by the manufacturer may be used.

40- 431760	Temperature measuring cell, NTC 1000 Ohm, 2 pcs./incubator
40- 431730	Cooler connection kit – (necessary if the incubator temperature is within 7 °C to ambient or below) – for max. 2 incubators
40- 419950	Cooler connection kit, with valve block, for max. 3 (4300) incubators
35- 100301	Water cooler / recirculator
40-431700	Sample cell rack, 16 positions
40-414735	Rack for indirect vials, 21 positions
40-431770	Set of test cells (4 pcs.)
40- 419899	Cleaning set
40-419200	Calibration standard (upon request)
30- 800241+	UPS 1500VA, uninterruptible power supply
30-800242	Smart-UPS 2200VA, uninterruptible power supply

6.2 Consumables

Sample cells and accessories as well as exchange parts (see price list)
Media (see price list)

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7 Certificate of Conformity

Included with the instrument.

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8 List of figures

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Part 2: Microbiological Manual

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1 Introduction

1.1 Basic principles of impedance analysis

Microbial metabolism utilises nutrient substrates which for the most part consist of higher molecular compounds (proteins, peptides and carbohydrates), producing lower molecular, charged decomposition products.

These newly formed, charged compounds and/or their dissociation products increase the conductivity of liquid nutrient media and lower their resistance.

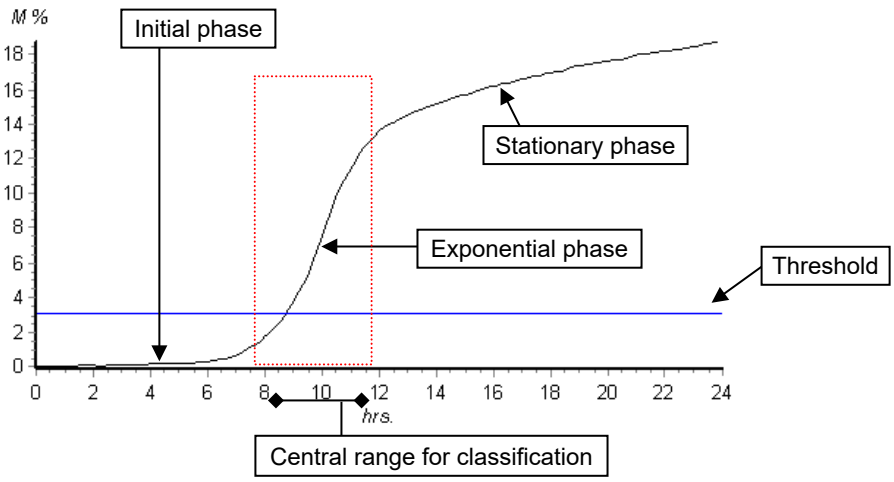
This decrease in electrical resistance can be measured using at least two electrodes introduced into the nutrient solution. If an AC voltage is applied to the electrodes, the decrease in the so-called **impedance** in the AC field can be measured.
The *BacTrac* measuring technology (see section 2.1) is based precisely on this principle, whereby the time lapse of the alteration of the ionic composition in the nutrient is recorded.

Despite the decrease in the actual measuring signal, positive measurement curves are displayed as a result of a standardisation process (showing the relative signal change in relation to the starting value). The resulting curves resemble typical microbial growth curves (fig. 1.1).

The typical *BacTrac* measurement curve can be divided into three characteristic ranges:

- Initial or adaptation phase.
- Exponential phase.
- Stationary phase.

Fig. 1.1: Typical impedance signal



During the **initial** or **adaptation phase** the microorganisms adapt their metabolism to the substrates present in the nutrient and begin exponential growth. The resulting ions cannot yet, however, be measured technically. As soon as the exponentially proliferating microorganisms have reached a concentration of approx. 10^6-10^7 CFU/ml of nutrient solution the measuring curves reach their characteristic turning point and then rise in a linear form during the **exponential phase**.

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The point of inflection of the curve and the earlier exponential phase, respectively, has the most relevance for the measuring technology.

As a result of the nutrient limitation or caused by the accumulation of toxic by-products, the microorganisms cease the metabolism and their exponential growth and there follows a transition to the **stationary phase**. The low molecular metabolites hardly increase at all and the measuring curve proceeds practically parallel to the time axis.

The typical measuring curve establishes more rapidly the more microorganisms are introduced to the sample under investigation in the nutrient solution. The formation of the measuring signal is thereby **indirectly proportional** to the bioburden load of the sample.

The higher the contamination rate of the sample is, the shorter is the length of time before the formation of the characteristic measuring curve.

The few microorganisms of only lightly contaminated sample materials – and the *BacTrac* is actually effective with a very small amount of microorganisms – require a longer period before reaching the above detection threshold (approx. 10^6-10^7 CFU/ml) than more highly contaminated samples. On the other hand, extremely microbiologically contaminated samples are therefore detected after a short analytical period.

1.2 Differences in comparison with standard plate count (classic microbiology)

Although impedance analysis is also a culture-based process (evaluation based on the assessment of proliferating microorganisms), it is fundamentally different to detecting microorganisms using the standard plate method (pour plates, spatula plates, drop plates, spiral plates).

In the plate process the microorganisms present in a sample are isolated via dilution so that when a suitable nutrient agar is introduced, followed by incubation to cause proliferation of the microorganisms, single, countable colonies are produced.

Once the microorganisms have proliferated to approx. 10^8-10^9 cells, typical colonies are produced which are visible to the naked eye. After a corresponding incubation period, which allows the microbes to proliferate and appear, the resulting colonies can be counted. The result is multiplied by the dilution factor to determine the sample contamination level.

As there is no way to objectively determine how many microorganisms originally formed a visible colony on the plate surface, the result is always given in “colony-forming units” (CFU).

The result of the plate method is therefore a **standardised reference value** for the microbiological contamination of a sample, which can never give the absolute number of proliferation-capable microorganisms in a sample.

Essential for the plate process therefore are the **dilution** of the microorganisms present in the sample to a countable range, the use of **semi-solid nutrients** (agar plates) and a **sufficient**

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incubation period (min. 24-72 hours) to enable the colonies to be observed. The process thereby involves a largely static end point.

Impedance analysis is a dynamic process, which determines the metabolic capacities of proliferating microorganisms. It is therefore not important whether microorganisms are present singly or in groups; **the metabolic capacity of each individual cell contributes to the overall metabolism** which in itself is responsible for the changing of the ionic composition of the nutrient.

This method not only predicts the number of microorganisms present in the sample, it also determines their level of activity, which is ultimately decisive in the spoiling potential of the microorganisms present in the sample.

1.3 Advantages of impedance microbiology

Using dynamic detection of metabolic processes to measure microorganism levels eliminates the need for dilution or separation of microorganisms, **which considerably reduces the sample preparation time**.

The liquid media used in impedance analysis is far better suited to the proliferation demands of microorganisms than the semi-solid agar media in the traditional process. The proliferation rate is accelerated and the **analytical times are reduced**. A further reduction of the analysis time is achieved via the **higher detection sensitivity**.

As signal changes can be determined via electrical measurement techniques once the microorganisms have increased to 10^6-10^7 CFU/ml (as mentioned in 1.1) in comparison to the plate method (10^8-10^9 CFU for a visible colony), the detection sensitivity exceeds that of the plate method by a factor of 1000.

In all, the total analytical time for impedance analysis rarely exceeds 24 hours. In most cases the **results are available within only a few hours** with the great advantage that the results are generally available earlier, the more contaminated a sample is.

Along with the simplification and **automation of documentation** the advantages of impedance analysis can be summarised as follows:

- Automation of a culture-based process.
- Significantly reduced detection time (within hours).
- Simplified sample preparation for quantitative determination.
- Saving on materials and working time.
- Increased sample throughput.
- Simple and comprehensive documentation.

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2 BacTrac measuring principle

2.1 Principles of the measurement technology

When microorganisms are introduced into liquid media, the result is proliferation of the microbes which decompose the higher molecular and mostly lowly or non-charged metabolites in the nutrient substrates. The resulting ions lead to measurable changes in the charge and electrical resistance in the nutrient.

When an AC voltage is applied via at least two electrodes immersed in the nutrient, this effect leads to a change in the impedance (= electrical impedance in the AC current field, measurement unit *Ohm, Ω*) and to **admittance** (= reciprocal value of the impedance, measurement unit *Siemens; S*).

In fact, the impedance (Z) represents a complex value and is defined in the measuring process by a capacitive component (C) and an ohmic resistance (R) via the following function equation:

$$Z = \sqrt{R^2 + (\frac{1}{2\pi FC})^2}$$

F... Frequency

Every increase in the admittance leads correspondingly to a reduction in the impedance and to an increase in current.

The microbial metabolism usually also leads to an increase in conductivity and capacitance and thereby to a drop in the impedance.

All concepts for the technical measuring of impedance, admittance, conductivity, capacitance and resistance are based on different measurement strategies but all parameters are influenced reciprocally. Of practical significance is the fact that the electrical signal consists of a conductive and a capacitive component and is frequency and temperature-dependant.

2.2 Direct impedance measurement

As already mentioned in section 1.1, the change in impedance of the nutrient is caused by the decomposition of nutrient substrates in the process of microbial metabolism. The nutrients in microbiological nutrient media are usually weakly charged or non-charged, but are transformed into highly charged compounds via the metabolism of the microorganisms, thereby increasing the conductivity of the nutrient broth. As a simple example, let us take the decomposition of glucose – a non-ionised substrate – into two molecules of lactic acid and the resulting increase in conductivity.

The lactic acid formed can be further metabolised by the uptake of three molecules of oxygen (O_2) to three molecules of CO_2. Three ion pairs of bicarbonate ions with a significantly higher conductivity are produced. Hydrogen ions, for example, are seven times more effective than sodium ions regarding their signal generation, whereby the buffer effect of the nutrient media is of great significance. The composition of the nutrient media is therefore a decisive factor in the quality of the results (see section 8).

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Greatly simplified, the *BacTrac* registers the net change in impedance in the nutrient broth at regular intervals.

2.3 Indirect impedance measurement

As well as direct measurement of impedance in the nutrient broth, the detection of carbon dioxide (CO_2) produced via the microbial metabolism provides the additional option of recording the activity levels of microorganisms. In this process the impedance change is not detected directly in the nutrient, rather it is the change in impedance of a potassium hydroxide solution caused by the uptake of the CO_2 formed.

The chemical reaction between CO_2 and the KOH solution is based on the following chemical equation:

$$CO_2 + 2\ OH^- \rightarrow CO_3^{2-} + H_2O$$

In this case, 1 ml of a 0.2 % KOH solution is placed in a special measuring cell (tightly sealed, with shorter electrodes) and the sample is introduced to the nutrient by means of a separate container into the measuring cell. The measuring cell is then tightly sealed and incubated. The sample vessel must not be completely gas-tight, otherwise the CO_2 created during the incubation period will not be able to escape.

All aerobic proliferating microorganisms produce CO_2 as the final product in the cell metabolism and are therefore suitable for the indirect method.

The microbiologically formed CO_2 is absorbed by the potassium hydroxide solution near the electrodes and the resulting production of potassium carbonate hereby increases the impedance of the solution. The measurement signal therefore indicates negative progress.

The indirect impedance measurement therefore represents a rapid and very simple process with pronounced higher sensitivity for the detection of CO_2 production. For the detection of yeasts and moulds in particular, whose changes occasionally hardly register in the direct method, the indirect method is far superior.

2.4 BacTrac signal development

The instruments of the *BacTrac* 4000 series are registering two specific impedance values for each single measurement. These values are displayed as **M-**value (media impedance) and **E-**value (electrode impedance) graphs (displayed as relative changes compared to a starting value) and are based on the **Impedance Splitting Method** as the measurement principle.

The relative measurement offers the advantage that all measurement curves have an identical starting point, thereby eliminating product specific fluctuations in the starting conductivity. The change in the M-value is therefore the relative change in the reduction of the media impedance (in percent) in relation to the starting value. The M-value mainly represents the conductive part of impedance (see section 2.1). It is directly influenced by the ionic composition of the growth media and sample used and therefore is not applicable to media of high conductivity since there signal changes can hardly be registered in the case of microbial growth.

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The proprietary impedance splitting measurement technology of the *BacTrac* instruments enables even the use of nutrient media with higher conductivity and therefore significantly broadens the possible applications. In addition to the M-value changes the E-value (electrode impedance) will be registered within the impedance splitting concept. For registration of the electrode impedance value the capacitive component of the complex impedance value Z (influence of the electrochemical double layer of the electrode) is of central importance. The way how to measure this electrode impedance value is the special characteristic of the *BacTrac* measurement technology.

The E-value will is not significantly influenced by the composition of the growth media and is therefore of central importance when media with high salt content (i.e. addition of salts as selective components for the detection of pathogens) will be used.

- ☞ For E-value registration the coverage of the whole surface area of the electrodes is necessary.

- ☞ For M-value registration it is only important that all electrodes are in contacted by the growth medium. Approx. 0.5 ml of growth media for reusable glass measuring cells and approx. 1.0 ml for disposable polystyrene measuring cells are sufficient.

- ☞ For the indirect measurement method (CO_2) the E-value is of no importance.

The instruments show the **relative change in the measurement signal**, displayed in **%M** (M= media impedance) and **%E** (E = electrode impedance) for the duration of the set measurement period, whereby the measured variables are recorded at a 10-minute (or longer) interval.

Because of the high-resolution measurement electronics, changes of 0.5-1 %M as well as of 2-3 %E can be considered being significant.

Compared to the M-value result, a bit higher deviations are tolerable for the E-value which is due to the high sensitive registration technology.

Figure 2.1 shows a typical measurement signal.

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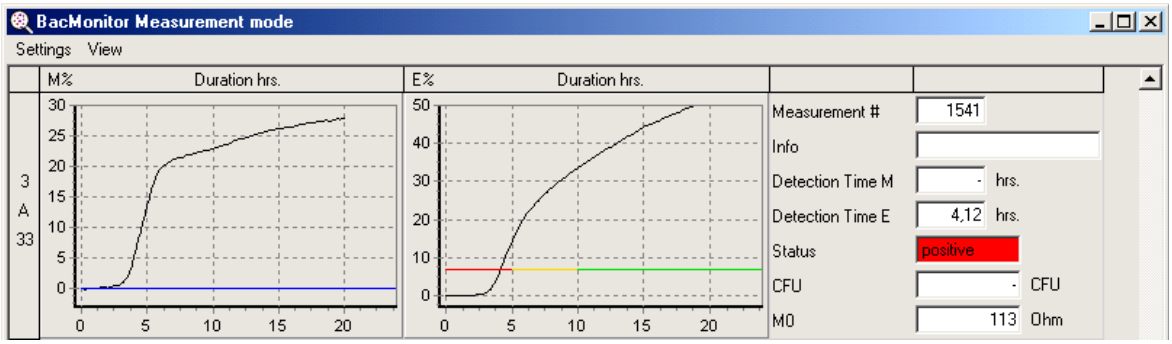
Fig. 2.1: Typical *BacTrac* measurement curve



- **Measurement #**
  Number of actual measurement as stored in database. This number is selected automatically from the system as soon as warm-up time has expired and the measurement is started.

- **Info**
  The content of Info field 1 is displayed in this window.

- **Detection time M**
  Time from start to surpassing the threshold of the M-signal.

- **Detection time E**
  Time from start to surpassing the threshold of the E-signal.

- **Status**
  Actual measurement or evaluation status of the sample.

- **CFU**
  Colony forming unit (germ count) according to a pre-selected calibration.

- **M0**
  Impedance value at start of the measurement.

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3 Commissioning the devices and setting the measurement parameters

3.1 Commissioning the devices

3.1.1 Installation of the measurement and control software

The *BacWin* software is delivered on CD-ROM, providing an installation assistant that will guide you during the installation protocol after the installation process has been activated. The software will be installed in a directory called "SY-LAB\BacWin2" of your local Windows system drive. Besides the main applications BacMonitor and "BacEval plus", dongle drivers, Firebird SQL database components and a Firebird ODBC driver will also be installed silently in the background.

At the end of the installation process the service for creation of a new database (CreateDB) will be started automatically. If no existing *BacWin* database is found, a new one will be created automatically. If an existing database is found, the user will be informed and furthermore asked if it shall be used as the main database. (See section 3.1.3 which describes the manual creation of a database.)

☞ Administrator rights are necessary to install the software.

The *BacWin* software is installed on the system volume of the computer in the directory "C:\SY-LAB\BacWin2", assuming drive C: to be the Windows system volume. (For software versions up to 2.42 the standard path is "C:\Program Files\SY-LAB\BacWin2".)

If the PC system has been provided by SY-LAB Geraete GmbH, the *BacWin* software has already been pre-installed and is ready for use. The delivered CD-ROM can be used to reinstall the software if necessary.

If the PC system is not provided by SY-LAB Geraete GmbH, the following hardware requirements must be fulfilled:

- Operating system: Windows 7 Professional, Windows 8.1 Pro, Windows 10 Pro
- Processor: Pentium IV or compatible, clocked at 2.0 GHz min.
- RAM: Min. 1 GB.
- Hard disc: Min. 10 GB.
- CD ROM drive: Required for installation.
- Serial ports: One per each incubator (Not needed if devices feature USB port).
- USB ports: One for the Hardlock dongle (For 4300 instruments with USB port (model 4310) one additional each).
- Min. one free slot for serial port multi I/O card (option).
- The use of an uninterruptable power supply (UPS) for *BacTrac* and PC is strongly recommended! One *BacTrac* unit including the PC can be sustained by a 2200 VA unit for approx. 30 minutes.

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☞ **To run the *BacWin* software it is necessary to connect the Hardlock dongle to a USB port of the PC. This dongle is delivered together with the software CD-ROM and serving as a copy protection.**

3.1.2 Installation of the BacTrac incubators (hardware)

Connect the *BacTrac* devices with the delivered main cord to your power line. Using the connection cables provided (either 9-9 null modem or USB in conjunction with model 4310), connect the incubators to a free port of the PC each.

☞ The instrument will be delivered with a type F plug (Schuko) power cord. If wall sockets which are not compatible with this plug are in use in your country you will need a local power cord to substitute the included one. Alternatively a universal country adapter can be used.

If you want to connect more incubators to a PC than free ports are available, an optional interface card is necessary. This plug-in card must be installed into a free slot of your PC together with the corresponding device driver for the operating system used.

☞ A maximum of 12 incubators connected to one PC will be supported by the *BacWin* software.

☞ During the installation process of the *BacWin* software the corresponding USB drivers for *BacTrac* incubators of type 4310 are installed as well. From this moment forth the instruments will be detected correctly by the operating system.

After the *BacTrac* incubators have been connected to the PC as well as to the power line the devices are ready for use and can be switched on using the power (I/O) switch on the back.

Immediately after the incubators have been switched on, they are heated to the nominal temperature (37 °C for a brand-new instrument). To change the nominal temperature for *BacTrac* 4100 and 4110 use the up and down buttons next to the device display. For *BacTrac* 4300 the temperature has to be set via the *BacWin* software (see section 3.4.1).

☞ *BacTrac* 4100/4110 incubator with internal device software no. >8.0: The final adjustment of the measurement temperature will be performed identical to the *BacTrac* 4300 via the measurement software using the function “Device settings” in the “Settings” menu of the BacMonitor overview window (see section 3.4.1).

3.1.3 Creation of a database

During installation a system database will be created automatically.

To manually create a database, please follow this step-by step procedure:

1. Start service application CreateDB from program group *“BacWin2”*.
2. Select button "Create new database“.
3. Specify database file name with „Specify a new database file“. (A standard “Save” dialogue is shown.)

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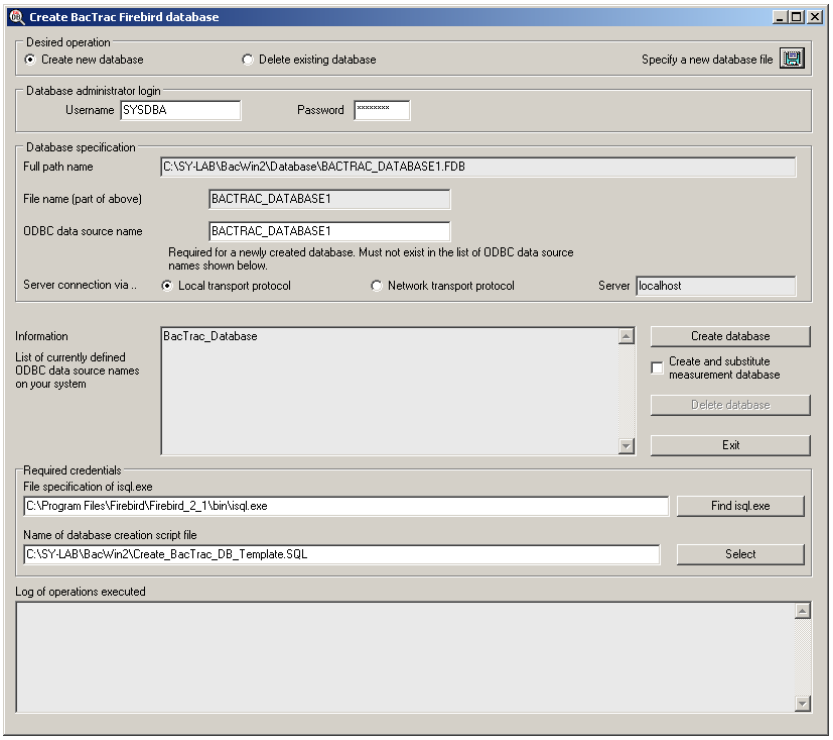
4. Specify username and password of database administrator. If you have not changed the user configuration of your Firebird installation (yet), enter the username of the database administrator **“SYSDBA”** and the password **“SyFbBMX!”**. (*BacWin* 2.60 and older versions have not changed this password while installation, therefore it can also possibly be **“masterkey”**.) (If your login fails, either the credentials of the database administrator **“SYSDBA”** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.) (In all input fields where a password is to be entered it is only shown as stars, see fig. 3.1.)
5. Create database by clicking button „Create database“.

Fig. 3.1: Service application CreateDB



For information purposes the names of the existing ODBC data sources are displayed in a small list box to prevent the operator from selecting a name that is already in use.

☞ **SY-LAB strongly recommends setting up the database on a volume with NTFS file system. Along with the FAT file system that is still in use this file system provides increased security and data integrity in the event of power cuts and is essential to ensure that the database can withstand power cuts of this kind.**

☞ It is also recommended that the database is not installed on a drive or in a directory where the Windows option “compress” is used.

☞ Because of system performance the database has to be created on a local drive. The usage of a network drive is not recommended.

☞ Databases are not properly deleted by deleting the associated files! Instead also use the application CreateDB for this purpose. Deleting works in an analogous manner

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to setting up the database by choosing „Delete an existing database“. The file name needs to be entered in an “Open” dialogue along with the username and password of the database administrator.

3.1.4 Updating an existing database to the latest format

When the *BacWin* software is revised and new features get implemented, sometimes a change of a database structure becomes necessary. Therefore, databases created with earlier versions must be updated. For this reason the application UpdateDB is provided.

To update an existing database please follow this step-by step procedure:

1. Start service application UpdateDB from program group *“BacWin2”*.
2. Specify update script (currently “Update_BacTrac_DB_V205.SQL”).
3. Choose target database from list of available databases.
4. Enter username and password of database administrator. If you have not changed the user configuration of your Firebird installation (yet), enter the username of the database administrator **“SYSDBA”** and the password **“SyFbBMX!”** (*BacWin* 2.60 and older versions have not changed this password while installation, therefore it can also possibly be **“masterkey”**.) (If your login fails, either the credentials of the database administrator **“SYSDBA”** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.)
5. Click button “Start script” to start update.

3.1.5 Setup of automatic database backup

An automatic backup schedule can be configured using the application DBBackupService. It appears as a window and further as an icon in the Windows notification area.

The following settings should be configured here:

1. Backup intervals.
2. Database to be backed up (usually located in C:\SY-LAB\BacWin2\Database).
3. Target directory for backup (can be an intranet drive).
4. Prefix for backup file (preset to “BACK_”).
5. Suffix for backup file. (Usually “.fbk” is entered automatically.)
6. Database login information. If you have not changed the user configuration of your Firebird installation (yet), enter the username of the database administrator **“SYSDBA”** and the password **“SyFbBMX!”** (*BacWin* 2.60 and older versions have not changed this password during installation, therefore it can also possibly be **“masterkey”**.) (If your login fails, either the credentials of the database administrator **“SYSDBA”** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.)

By clicking “Save” or “Save and exit” the settings are stored in an .INI file and become valid.

☞ To automatically start DBBackupService when the system is started a link to the corresponding application has to be put in the program group “Startup”.

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☞ The application backs up according to the defined time plan, regardless whether the BacMonitor is running (or has run in the meantime).

☞ A new file is created in the target directory at each backup time. If backups are scheduled to be carried out daily and these files are not deleted after a certain period of time, sooner or later some overflow could be produced.

3.1.6 Creating a manual backup (as required)

Besides the automatic backup function there is the possibility to create a manual backup anytime it is required. To do so start the application DBBackupCreator from the program group *"BacWin2"* and select the option "Backup".

Additionally the following information has to be provided:

- Database to be backed up (usually located in C:\SY-LAB\BacWin2\Database).
- Target directory for backup file. (It can be a network drive, too.)
- Database login information. If you have not changed the user configuration of your Firebird installation, enter the username of the database administrator **"SYSDBA"** and the password **"SyFbBMX!"** (*BacWin* 2.60 and older versions have not changed this password while installation, therefore it can also possibly be **"masterkey"**.) (If your login fails, either the credentials of the database administrator **"SYSDBA"** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.)

By clicking the button "Start" the backup is being created.

3.1.7 Restoring a backup

If the existing database is corrupted and cannot be accessed anymore by the *BacWin* software it is required to restore its latest backup.

☞ **Restoring a backup permanently overwrites the existing database. All data recorded since the latest backup was performed will be lost!**

☞ Before restoring a backup the applications BacMonitor and "BacEval plus" must be closed if they are currently running.

Start DBBackupCreator from the program group *"BacWin2"* and select the option "Restore".

Additionally the following information has to be inserted:

- Database to be overwritten by selecting correct path (usually C:\SY-LAB\BacWin2\Database\BACTRAC_DATABASE.FDB).
- Destination of latest backup file.
- Database login information. If you have not changed the user configuration of your Firebird installation, enter the username of the database administrator **"SYSDBA"** and the password **"SyFbBMX!"** (*BacWin* 2.60 and older versions have not changed this password while installation, therefore it can also possibly be **"masterkey"**.) (If your

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login fails, either the credentials of the database administrator **"SYSDBA"** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.)

By clicking the button "Start" and confirming the warning message the backup is carried out.

3.1.8 BacWin software – preparation of instruments for the measurement

The *BacWin* software consists of two parts:

1. *BacTrac* measurement application **(BacMonitor)**.
2. *BacTrac* data evaluation application **("BacEval plus")**.

The *BacTrac* measurement application **(BacMonitor)** is used for the detection of measured variables and data management and as such it represents the central part of the software. With this application you can install and/or activate individual incubators, adjust all measurement parameters, and manage ongoing measurement cycles.

At the start of the application you will see the BacMonitor overview window. Before adjusting measuring and analysis parameters, selection of the language and alignment (installation) of the already connected incubators to the individual serial ports becomes necessary.

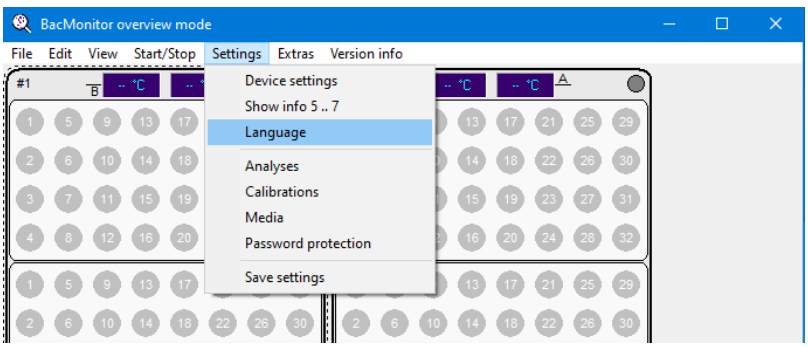
3.1.8.1 Selecting a language

The application language can be chosen from the BacMonitor overview window by selecting "Language" in the "Settings" menu. The corresponding .LNG files can be found in the *BacWin* application directory and applied in any of the *BacWin* applications.

☞ *BacWin* version 2.60 and higher: If a certain language shall be loaded while some application starts the checkbox for "Default language" has to be ticked while the appropriate language file within the "Open" dialogue is being chosen.

☞ Until *BacWin* version 2.59: If a certain language shall be loaded while some application starts the function "Save settings" from the menu "Settings" must be selected after the appropriate language file within the "Open" dialogue has been chosen.

Fig. 3.2: Language selection



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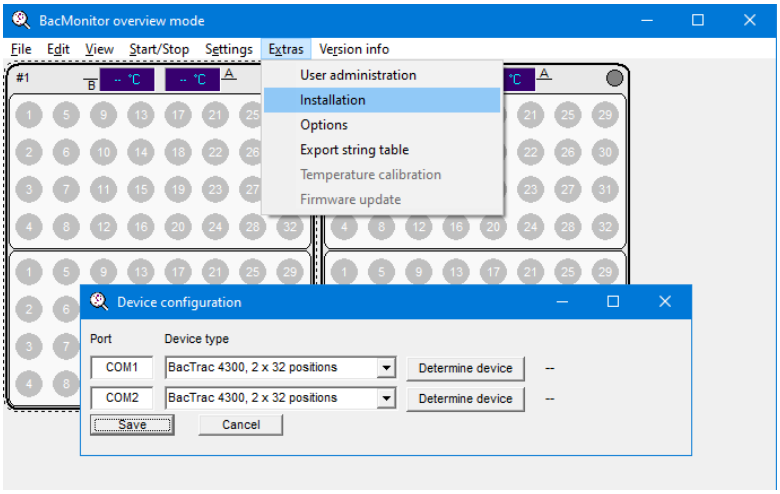
☞ If the language has been chosen but not defined as default setting and no other settings have been saved yet, all applications will run in English language when they are started.

3.1.8.2 Serial port alignment (device configuration)

Alignment of serial ports to the type of connected incubators will be performed in the BacMonitor overview window by selecting “Installation” of the “Extras” menu. The “Device configuration” window appears and all available COM ports will be displayed. Using the pull-down menu the appropriate device type can be aligned to the COM port selected.

Fig. 3.3: Device configuration



After clicking the button “Determine device” the serial number and/or the internal software version of the connected instrument will be automatically displayed.

☞ For a proper determination the instruments must be connected to the PC and powered on (see section 3.1.2).

After all incubators have been aligned with the correct instrument types on the respective COM ports the configuration must be stored by clicking the “Save” button.

☞ Device installation in BacMonitor is only necessary while the initial commissioning of the equipment is taking place or when incubators have been added resp. removed.

☞ The device configuration can only be modified when no measurements are running (i.e. all devices must be in stop-mode).

☞ If the device configuration is being modified, all specific parameters (incubation temperatures and parameters applied to individual measuring positions) **for all devices must be set anew. This is necessary even if the old parameters are partly viewable!**

☞ (Re-)saving the device configuration causes the first device (#1) to be focused when available, regardless if another device was focused before or not.

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☞ For devices of type 4310 (featuring a USB interface) a COM port is assigned to each, when the instrument is connected to the PC for the first time. The alignment is the same as with instruments connected via serial port.

3.1.8.3 Focusing a device

By default the first device is automatically in focus. Focusing a device is necessary when operations are to be performed which shall be applied to a specific device.

Click on the frame of the device which shall get the focus with the left mouse button. If the corresponding setting (see section 3.1.8.4) has not been changed yet, the focus will be shown as a dashed outline of the respective device (fig. 3.4).

☞ If a focused inactive device is being hidden (see section 3.1.8.5), none of the devices shown is focused.

3.1.8.4 Changing the display mode for a focused instrument

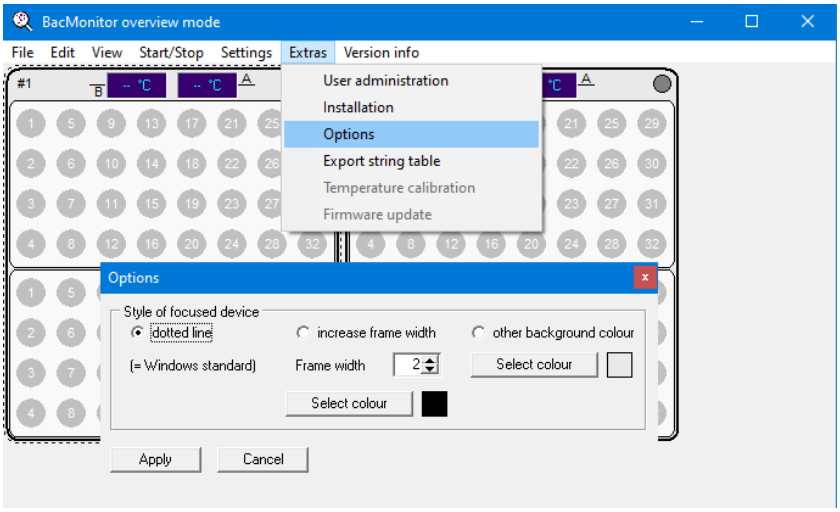
The colour and width of the frame which displays the focus can be changed by choosing “Extras/Options” in the BacMonitor overview window. In addition it is possible to change the background colour of the respective focused instrument (fig. 3.4).

3.1.8.5 Hiding inactive devices

Using the function “View/Hide inactive devices” it is possible to hide incubators which are currently not in use.

Hidden instruments can be made visible on demand by using the same function again.

Fig. 3.4: Display mode of focused instruments



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3.1.8.6 Internal software updates

On demand, an update of the *BacTrac* 4300/4310 internal software can be performed.

☞ This function may be utilised only when requested by the manufacturer or an authorized maintenance engineer, and only when the corresponding image file has been provided.

☞ A firmware update shall be performed only if the respective incubator is not running a measurement (= stopped).

☞ To view the version number of the internal software, double-click into the temperature display frame of the corresponding instrument or focus it and activate the function "Settings/Device settings".

3.1.8.6.1 BacTrac 4300

The latest firmware version can be found on the software CD in the folder Utility\Subsystem BT4300 V1.xx. At the moment the version is V1.90p. Check "Settings/Device Settings" for the current firmware version of your device(s). If the version displayed after the serial number is older than 1.90p and update is strongly recommended.

Using the function "Extras/Firmware update" within the BacMonitor overview window the firmware (= internal software) of the incubator currently focused can be updated. After this function has been activated the program image to be run has to be selected. The update will then start automatically.

After the process is completed the incubator has to be power cycled using the I/O switch on the back. Wait at least 60 seconds before switching the instrument back on. In this way the updated internal operating system will be loaded and run.

3.1.8.6.2 BacTrac 4310

The latest firmware version at the time of the release of your BacWin 2 software package will be put into the directory C:\SY-LAB\BacWin2\ImageLoader at the time of installation. Look for a filename starting with "BT4310" and check the version (three digits after the V in the file name). Now check Check "Settings/Device Settings" in BacMonitor for the current firmware version of your device(s). If the installed version is older than the version of the update file, it is strongly recommended to perform an update.

- Switch off the instrument before performing the update.
- Open directory C:\SY LAB\BacWin2\ImageLoader and start the application "ImageLoader_BT4310_4620".
- Select "file" on the left and browse C:\SYLAB\MicroTrac2\ImageLoader\BT4310_4620.img.
- Select the correct COM port on the right, the circle should go to green now.
- Switch on the instrument, the firmware upload starts automatically.
- When the upload is finished (progress bar is completely filled), close the program.

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3.2 Starting the instruments

The menu “Start/Stop” within the BacMonitor overview window is used to activate the connected incubators either individually (“Start focused instrument”) or all at once (“Start all instruments”).

☞ *BacTrac* 4100/4110: After the instrument is started, a window asking for confirmation of the set incubator temperature will appear (fig. 3.5).

The device status display (= circle in the right upper corner of the incubator displayed in the BacMonitor overview window) will change its colour to yellow (initialising). After the initialisation cycle has been completed the instruments are ready for insertion of samples and the device status display turns into green.

☞ After selection of “View/Legend” in the BacMonitor overview window all the different symbols for the device status display, position display and error status which are marked with coloured status display and measuring positions can be viewed.

3.3 BacMonitor “watchdog”

Since *BacWin* version 2.42 a “watchdog” monitoring utility is included in the software package. Every time one or more *BacTrac* devices have been started the “watchdog” is also started automatically and monitors the status of BacMonitor. In case that BacMonitor does not respond or locks up unexpectedly during measurement the “watchdog” automatically restarts the application, restoring the prior status and continuing the measurement. As soon as all *BacTrac* instruments have been stopped the “watchdog” is shut down.

☞ Note that shutting down BacMonitor via the menu and confirming the warning message by clicking the button “Yes” stops all instruments and thus irreversibly concludes all ongoing measurements.

If the PC is shut down or restarted unintentionally during measurement the “watchdog” shuts down, too. In this case the measurement must be resumed as soon as BacMonitor is restarted manually by the user. This process reactivates the “watchdog”.

To ensure an automatic restart with continuation of measurement after a power loss the following steps have be followed:

- A shortcut to BacMonitor is present in the program group “Startup”.
- The PC is configured to boot automatically when a blackout occurs.
- An automated user login to Windows is activated.

In any case the PC should be connected to an uninterruptable power supply since a proper restart after a power blackout cannot be guaranteed.

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3.4 Setting measurement parameters

To perform a measurement the following parameters must be aligned to the *BacTrac* incubators and measurement positions to be used:

- Incubation temperature.
- Duration of the measurement.
- Sample warm-up time within the instrument.
- Thresholds.
- Automatic evaluation (optional).

☞ If these parameters for the first measurement are not selected until the end of the warm-up time a contact error will appear and no measurement curve will be displayed.

☞ Changes of settings on positions which are already loaded with measuring cells are restricted to the warm-up phase. Duration, drift compensation, “DropStop” and sample ID can still be changed even during ongoing measurements.

☞ If a new measurement cell is inserted into an empty incubator position without changing the analysis parameters the measurement will continue using the “old” parameters stored for the previous measurement. In contrast previous sample ID was already cleared when the measuring cell was removed.

☞ It is necessary for the user to identify himself/herself by selecting his/her user ID from the drop-down list to enter measuring parameters and sample labelling information.

3.4.1 Setting the BacTrac incubator temperature and measurement interval

The incubation temperature has a big impact on the generation time of microorganisms with direct influence on enzyme activities and impedance signal generation and is therefore of real importance in impedance analysis.

For comparability and reproducibility reasons, keeping constant incubation temperature is not only important during an analysis but also to compare results obtained on different days.

Selection of the function “Settings/Device settings” in the *BacTrac* overview window will open another window for setting temperature and measurement interval for the focused incubator (fig. 3.5).

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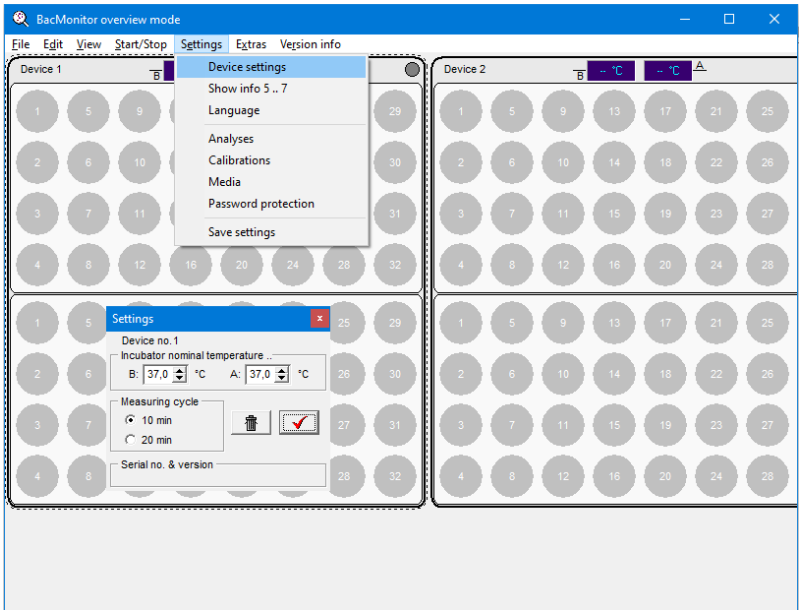
Fig. 3.5: Setting the incubator temperature and measurement interval



- ☞ *BacTrac* 4300/4310: In the lower part of the incubator temperature setting window the serial numbers and the internal (incubator) software version will be displayed.

- ☞ *BacTrac* 4300/4310: Please note that the temperature has to be set separately for incubators A and B.

- ☞ Incubator A is the one on the far end next to the blue display casing and its temperature can be adjusted via the right temperature display frame.

- ☞ Incubator B is the one next to the user and its temperature can be adjusted via the left temperature display frame.

The desired reference temperature is adjusted using the arrow buttons or the PC keyboard.

- ☞ After initial switching on of the instrument, the incubator needs approx. 30 minutes to adjust its temperature and keep it constant. During this time no measurement cells should be inserted.

- ☞ If the temperature is being reduced, reaching the new temperature may take longer if no active cooling device is connected via the adapters on the back side of the instrument.

The measuring cycle time (= interval time) defines the time span after every position will be measured periodically. A standard value of ten minutes is recommended. Only for measurements lasting longer than 72 hours this value might be increased to 20 minutes to record fewer individual measurement points and not allocate any unnecessary database memory.

After entering the new parameters have to be confirmed by clicking the "✓" button. When selecting the "Cancel" button, the last changes are discarded and the software reverts to the temperature used for the last measurement.

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☞ *BacTrac* 4100/4110 incubator with internal device software version <8.0: The set reference temperature will not be sent to the incubators because this function is not supported. A warning message indicating that the temperature has to be set directly on the incubator will appear after the instrument has been activated using the "Start" function.

☞ *BacTrac* 4100/4110 incubator with internal device software no. >8.0: A confirmation window for the set reference temperature will appear after the instrument has been activated using the "Start" function.

☞ To close this window a confirmation by clicking the "OK" button is necessary.

The respective current temperature is shown both on the display of the individual incubators and in the temperature section positioned next to the incubator # in the BacMonitor overview window.

In addition, for individual incubator positions there is an opportunity to determine the consistency of the temperature progress for the length of individual measurements by using a temperature measuring cell (optionally available accessory).

☞ A stable temperature can only be achieved if the difference between the set *reference* temperature and the ambient temperature is >6 °C. Otherwise active counter cooling is required via the cooling connector on the back of the device, using a thermocirculator to achieve the required temperature setting.

3.4.1.1 Calibration of the temperature sensors (BacTrac 4300/4310)

If the temperature measured within individual *BacTrac* measurement cells by the use of a calibrated thermometer is out of specification (set temperature ±0.5 °C), the internal temperature sensors of the *BacTrac* can be recalibrated.

Required materials:

- A calibrated thermometer.
- Two measuring cells filled with water.

☞ For the most precise calibration the use of an electronic thermometer and an overnight temperature adaptation time for the measurement cells used for the calibration is recommended.

To perform the temperature calibration, please follow the instructions below:

1. Turn on the device.
2. Start the application BacMonitor.
3. Insert the measuring cells filled with water into each position 29 of both incubators.
4. Put the sensor of your calibrated thermometer into the control cell of the first incubator and close the cover as far as possible.

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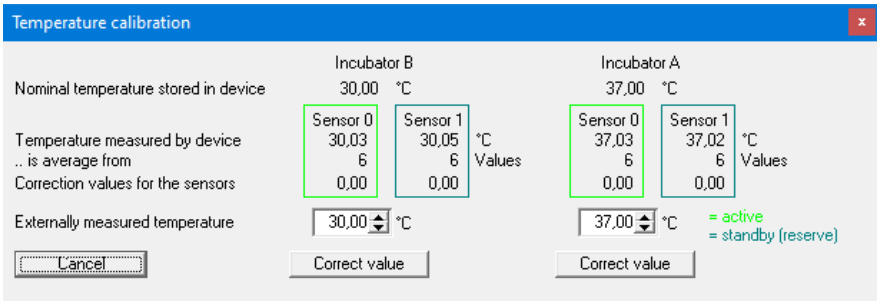
5. Let the device and the measuring cell reach a stable state of temperature. SY-LAB recommends waiting at least one hour after switching on and inserting the measuring cells, so that the temperature will be distributed evenly across the blocks and also the measuring cells have reached their temperature wanted.

   ☞ For the calibration the measurement temperature most frequently used should be selected.

6. Select the function "Extras/Temperature calibration" in the BacMonitor overview window and enter the password *SyTmpCal.* A new window (fig. 3.5) appears.

Fig. 3.6: Temperature calibration window



   ☞ Both *BacTrac* incubation blocks are equipped with two temperature sensors each, one of them is the active sensor (which is used for the temperature control), the other one is on standby in case the first sensor is defective.

7. Set the "Externally measured temperature" field to the reading of your thermometer and click the "Correct value" button. In turn, the fields ".. is average from" will be reset to zero, and the correction values for the sensors will be recalculated. As a consequence the "Correct value" button is greyed out until six consecutive measurements are available again.

   ☞ The value of the standby sensor will be transmitted only in measuring pauses, so eventually it is necessary to wait until the measurement run has finished to obtain the six readings required.

8. Repeat the procedure from step 4 on for the second incubation block in which the other measurement cell is located. After that, the temperature calibration is finished.

   ☞ It is not necessary that the nominal temperature (obtained from the last measurement temperature setting stored in memory) is reached, but the block temperature must be in a steady state.

3.4.1.2 Using the temperature measuring cell

In the temperature measuring cell, the electrodes are connected to each other via two high temperature sensitive resistors.

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The temperature measuring cell is supplied ready-to-use filled with a thermally conductive compound and is inserted in any BacTrac measuring position (earlier versions had to be filled with 10 ml sterile A.dest. or a mixture of glycerine / A.dest. (50 / 50%).

For measuring parameters, the measurement duration and the duration of the warm-up phase must be entered for every position fitted with a temperature measuring cell. The recommended range for this is -5 %M to +5 %M. There are no other inputs necessary.

If there are fluctuations in the incubator temperature these are registered in the form of a change in the resistance values in the temperature measuring cell.

If the temperature rises, e.g. from 37 to 37.5 °C, then the measurement signal also rises by approx. 1.5 %. If the temperature falls from 37 °C to 36.5 °C the measurement signal from the temperature measuring cell also falls by approx. 1.5 %.

As a result of the specified temperature constancy of ±0.5 °C in the incubator, ±1.5 %M fluctuations in the measurement signal of the temperature measuring cell are normal. Greater fluctuations can indicate problems with the temperature controller and may influence the measurement result. These therefore require further clarification.

A change in the measuring signal of the temperature by a temperature measuring cell of approx. 3 % corresponds to an absolute change of temperature in the incubator of approx. 1 °C.

☞ In measuring cells filled with nutrient broth the influence of changing incubator temperatures in comparison to the temperature measuring cell represents a significantly lower (approx. 50 %) effect on the relative change of the measurement signal (1 °C difference ≈ 1.5 % signal change).

☞ Please note that the above values approximations only, as the composition of the nutrient plays an important part in the process.

3.4.2 Duration of the measurement

The duration of the measurement is set individually for every incubator position in the “BacMonitor measurement parameters” window (fig. 3.7).

To open the view use the function key F6 or select “View/Measurement parameters” in the BacMonitor overview window. Alternatively you can open the “BacMonitor measurement parameters” window by moving the mouse pointer outside the circle resembling a measurement position and double-clicking the left mouse button.

The duration should be entered before measurement cells are inserted but until the end of the warm-up phase for already started measurements by the latest.

Prolongation of the duration of the measurement for active measurements is possible.

☞ It is impossible to enter durations for active measurements that are less than the time span already gone since the end of the warm-up time.

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For most of the applications a measurement time of 24 hours is sufficient, with the exception of yeast and mould determination and sterility tests, where a minimum duration of 48 hours is recommended.

According to the pre-set duration, the ring diagram in the BacMonitor overview window shows the actual running time in relation to the total duration by filling up the circle with a different colour (blue).

3.4.3 Threshold values and impedance detection time

The measurement parameters must be set and entered for measuring cells already installed in the incubator before measurement commences and/or by the end of the warm-up phase.

The most significant criterion for the evaluation of the measurement results is the so-called **impedance detection time (IDT)**. It is defined as the period between the start of measurement and the moment at which the **threshold value** is reached. The threshold value is dependent on the medium and method used, but typically lies within the early exponential growth curve in the linear section of the measurement curve, shortly after the turning point.

The impedance detection time is therefore *the* central parameter and is a function of the germ load of the sample. It is further influenced by the activity of the microorganisms present and the composition of nutrients. The quicker exceeded threshold value resp. the shorter the impedance detection time, the higher will be the contamination level of the sample.

3.4.3.1 Selection and setting threshold values

In order to allow evaluation of measurement signals during a measuring process, a threshold value must be selected before the start of measurement, at least before the end of the warm-up phase.

The threshold value is selected in the “BacMonitor measurement parameters” window in the section “Evaluation”.

If there is no threshold value given for the required application, we recommend setting the threshold value to between 3-5 %M and 5-10 %E, respectively.

The lowest possible threshold value can be determined empirically via the investigation of several sterile samples. It should be set high enough so as not to be interrupted by negative growth signals (baseline). In the event that an unsuitable threshold value is set, this can be adjusted after the measurement process. Note, however, that this will also alter the impedance detection time.

Threshold values and other parameters need not be set separately when *BacTrac* software analysis settings already saved shall be applied and are accessed via the pull-down menu “Analysis”.

- ☞ To compare measurement results it is absolutely essential to use a common threshold value.
- ☞ For simultaneous alignment of measurement parameters to different positions see advanced copy possibilities described in section 3.4.9.

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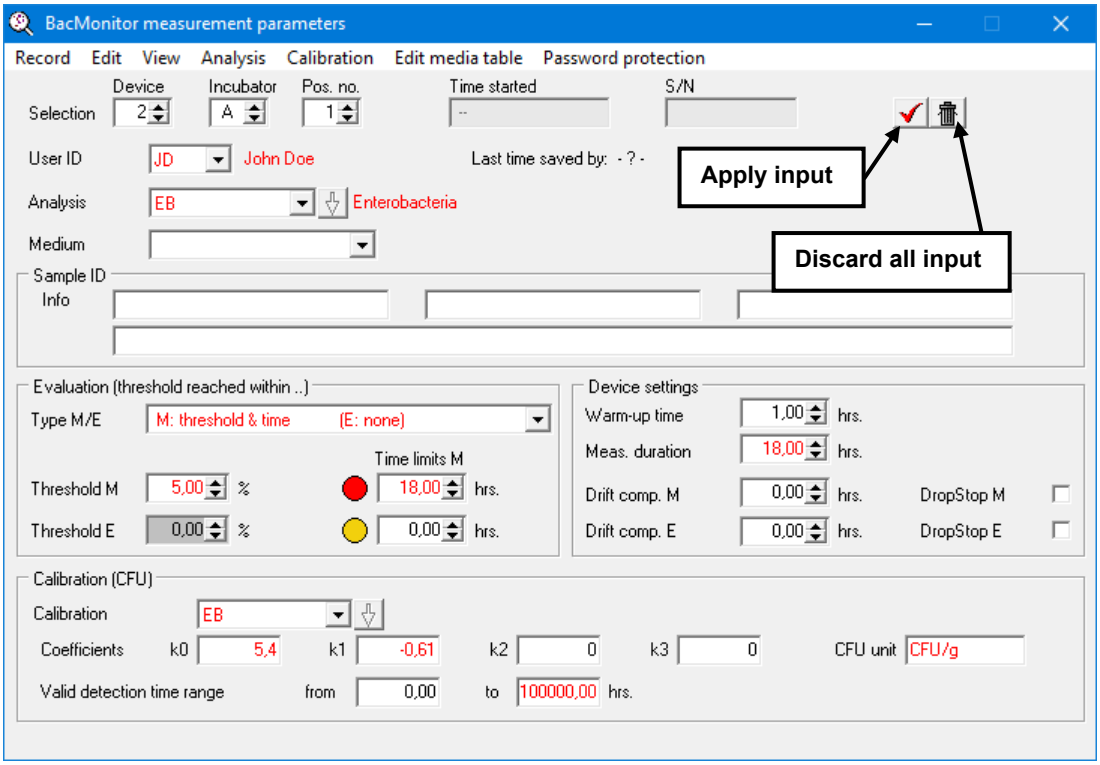
Fig. 3.7: BacMonitor measurement parameters



- **Device**
  By using the arrow buttons or entering the *BacTrac* device number directly, the respective instrument can be selected.

- **Incubator**
  By using the arrow buttons or entering the identification (A or B) directly, the respective *BacTrac* incubator can be selected. (For *BacTrac* instruments of types 4100 and 4110 this field has no function.)

- **Pos. no.**
  By using the arrow buttons or entering it directly, the individual position number of the respective incubator can be selected.

- **User ID**
  By using the pull-down menu a user who is already registered in the database can be selected.

- **Analysis**
  By using the pull-down menu or the function "Select" from the menu "Analysis" parameters from analyses already saved can be selected. This field can be accessed only if a registered user ID has been selected before. To fill in parameters of an analysis already selected it is necessary to click the blue "🡻" button next to the analysis chosen. The final acceptance of parameters selected for the position demands a confirmation by using either the button with the red checkmark or the function "Save change(s) to database" from the menu "Edit". If an analysis selected is not confirmed within five seconds the field will reset to "– no analysis selected –" by itself.

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- **Medium**
  By using the pull-down menu the medium used and the temperature adjusted can be selected. Thus the application compares the measured value with the nominal M0 value at the beginning of the measurement and shows the error message “M0 exceeded” in case of a distinct deviation.

- **Sample ID**
  Four text fields for entering the sample ID are available.

- **Evaluation**
  By using the pull-down menu, the arrow buttons or entering directly parameters for an automated evaluation (red/yellow/green) according to pre-defined TVC limits or presence/absence can be used. (See section 3.4.5 for details.)

- **Calibration**
  By using the pull-down menu or the function “Select” from the menu “Calibration” parameters from calibrations already saved can be selected. This section can be accessed only if an evaluation type (all except “X” and “Xrel”) has been selected before. Single input of coefficients can be performed, too. The acceptance and confirmation is carried out in an analogous manner to the selection of an analysis. (See section 6.6.1 for details.)

3.4.4 Warm-up phase

The warm-up phase or warm-up time is measured from the moment the measuring cell is placed in the incubator to the actual start of the measurement (signal recording).

This period is of great importance therefore since

a) it is necessary to adjust the measuring cells to incubation temperature. (The impedance analysis, as mentioned already, is temperature dependant and influences the measurement signals.)

b) only results with an identical warm-up time can be compared with one another. (As the microorganisms start to multiply immediately after being introduced to a suitable nutrient, the time consistency is especially important for comparative investigations.)

Once set, the duration of the warm-up phase should not be changed again, particularly for routine analyses.

3.4.4.1 Setting the warm-up phase

A standard warm-up phase lasts 60 minutes. During this time the set incubation temperature in the measuring cells is guaranteed to be reached.

The warm-up time is to be entered in the section “Device settings” of the “BacMonitor measurement parameters” window (fig. 3.7).

☞ Pre-filled measuring cells or nutrient media which have been kept in a fridge must be brought to at least room temperature before use.

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☞ If no other warm-up time has been set when the measuring cells are put in place, the default warm-up time of 60 minutes will apply.

☞ The lowest possible limit for the warm-up time is 60 minutes.

☞ The warm-up time must be set before a measurement cell is inserted into the incubator (e.g. for the empty position), otherwise the default warm-up time of 60 minutes will be used for the measurement. A prolongation of the warm-up time in such a situation can only be performed after the set measurement time is expired and the measurement is still active.

☞ Any change in the measurement parameter window appears in red colour and needs to be confirmed by the function "Edit/Save change(s) to database" before it will be used.

3.4.5 Evaluation

Analysis results can be simultaneously classified by the *BacWin* software.

Automatic classification dramatically simplifies evaluation. It is possible to divide the measurement results into two categories (red/green) or three categories (red/yellow/green).

The red/green classification allows very simple colour differentiation between contaminated or uncontaminated samples, along with the allocation of quantitative measurement results (when using calibrations, see section 6) to reference warning and limit values (e.g. values from the three classes) in the form of:

- green = complies = negative
- yellow = suspect/borderline
- red = does not comply = positive

3.4.5.1 Selection and setting evaluation criteria

The evaluation criteria are basically determined using time thresholds. If a single time threshold has been entered in the "measurement parameters" window (fig. 3.7) for the colour red, results are classified into two categories (red/green).

In this case the sample is classed as **green = negative**, if the given threshold is interrupted by the measurement signal after the time threshold given for red.

If detection takes place within the set time threshold the result will be classed as **red = outside specification/positive**.

If a time threshold has been set for yellow different from that set for red, classification is into three categories:

- Measuring positions marked **green** are those whose impedance detection time (IDT) is greater than the threshold time given for yellow.

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- Samples are evaluated as **yellow** when their IDT is greater than that for red, but lower than the threshold time given for yellow.

- All samples whose detection time is lower than the value set for red, are evaluated as **red**.

These colours can be very simply allocated to germ count values for quantitative applications on calibrated systems. (To find out how to allocate germ counts and determine time thresholds for evaluation purposes, see section 6.)

☞ When selecting the time thresholds for a 3-category evaluation, the smallest value must always be assigned to red.

3.4.5.2 Evaluation types

Automatic evaluation can only be performed **either** on M-value **or** E-value. There is also an option that evaluation is performed on M-value but in addition a defined E-threshold must be reached and vice versa.

Eleven different evaluation types are therefore possible:

a) No evaluation
No automatic evaluation (red/yellow/blue) will be performed during an ongoing measurement. The diagram circle remains blue for the whole measurement time. Detection times will be calculated according to the pre-set thresholds for M and E respectively but will not be saved to the database.

b) M: threshold & time (E: none)
Evaluation will be performed on M-value with respect to the pre-set threshold and time limits for M.
If the M-threshold will be reached, the set time limits for M will be applied to align the red/yellow/green colour to the measurement position.

☞ Using this evaluation type setting a threshold for E is not possible since it is not considered for sample evaluation.

c) E: threshold & time (M: none)
Evaluation will be performed on E-value with respect to the pre-set threshold and time limits for E.
If the E-threshold will be reached, the set time limits for E will be applied to align the red/yellow/green colour to the measurement position.

☞ Using this evaluation type setting a threshold for M is not possible since it is not considered for sample evaluation.

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d) M+: threshold & time, E: threshold

Evaluation will be performed on M-value with respect to the pre-set threshold and time limits for M only if additionally the pre-set threshold for E will be reached.
Colony counts will be calculated according to calibrations performed on M-values but only if the E-thresholds are reached.

☞ The evaluation will be performed only if both criteria are met, otherwise no counts will be calculated and no colour will be aligned for the corresponding positions.

☞ This evaluation type is only applicable to a small range of applications.

e) E+: threshold & time, M: threshold

Evaluation will be performed on E-value with respect to the pre-set threshold and time limits for E only if additionally the pre-set threshold for M will be reached.
Colony counts will be calculated according to calibrations performed on E-values but only if the M-thresholds are reached.

☞ The evaluation will be performed only if both criteria are met, otherwise no counts will be calculated and no colour will be aligned for the corresponding positions.

☞ This evaluation type is only applicable to a small range of applications.

f) X: M or E (threshold), time: any

Evaluation will be performed on M-value or E-value with respect to the pre-set threshold and time limits for M or E, depending on which one was crossed first.
If the M- or the E-threshold is reached, the set time limits will be applied to align the red/yellow/green colour to the measurement position.

g) M2: cells with 2 pins (no E-Value)

Evaluation will be performed on M-value with respect to the pre-set threshold and time limits for M.
If the M-threshold will be reached, the set time limits for M will be applied to align the red/yellow/green colour to the measurement position.

☞ Using this evaluation type setting a threshold for E is not possible since it is not measured.

☞ This evaluation type should be chosen only if measuring cells with two electrodes (instead of four) are used.

h) M2rel: 2 pins, 1st deriv. (no E-Value)

Evaluation will be performed on M-value with respect to the pre-set threshold and time limits for M.
The first derivative of the M-graph (M') will be shown and evaluation is based on this graph crossing the threshold, therefore the original graph is evaluated based on its slope not its amplitude. The original graph will be shown in blue colour for reference.

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☞ Using this evaluation type setting a threshold for E is not possible since it is not measured.

i) Mrel: 4 pins, 1st deriv.
Evaluation will be performed on M-value with respect to the pre-set threshold and time limits for M.
The first derivative of the M-graph (M') will be shown and evaluation is based on this graph crossing the threshold, therefore the original graph is evaluated based on its slope not its amplitude. The original graph will be shown in blue colour for reference.

☞ Using this evaluation type setting a threshold for E is not possible since it is not measured.

j) Erel: 4 pins, 1st deriv.
Evaluation will be performed on E-value with respect to the pre-set threshold and time limits for E.
The first derivative of the E-graph (E') will be shown and evaluation is based on this graph crossing the threshold, therefore the original graph is evaluated based on its slope not its amplitude. The original graph will be shown in blue colour for reference.

☞ Using this evaluation type setting a threshold for M is not possible since it is not measured.

k) Xrel: 4 pins, 1st deriv., M or E (threshold), time: any
Evaluation will be performed on M-value or E-value with respect to the pre-set threshold and time limits for M or E, depending on which one was crossed first.
If the M- or the E-threshold is reached, the set time limits will be applied to align the red/yellow/green colour to the measurement position.
The first derivative of both the M-graph (M') and the E-graph (E') will be shown and evaluation is based on these graphs crossing the corresponding threshold, therefore the original graphs are evaluated based on their slope not their amplitude. The original graphs will be shown in blue colour for reference.

3.4.6 Further settings within the “measuring parameter” window

3.4.6.1 Sample identification

There are four separated fields for entering the sample ID. Using this, a separation in distinct columns within the result and export table is possible.

If no separation is necessary, all your sample ID (text and/or numbers) can be entered into the first area.

☞ To facilitate combined data searches, a structured data entry is strongly recommended (e.g. sample ID in field “Info1”, method in field “Info2”, manufacturer in field “Info3”, etc.).

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☞ Move the mouse pointer over a measurement position to display the content of Info 1. Long texts will be displayed abbreviated.

3.4.6.2 Drift compensation and "DropStop"

Both functions may have a severe impact on the signal generation and should therefore be selected a priori only in exceptional cases.

These functions have been implemented mainly for the correction of atypical measurement signals and should therefore be used only on demand during an ongoing measurement or during the evaluation of results (see section 5.2). Both functions can be selected for M- and E-values separately.

3.4.6.2.1 Drift compensation

Using that function the initial part of the curve until the pre-set time is deleted. Instead the curve will start from the set time at 0 % impedance. This function can be used to correct sudden atypical fluctuations of the measuring signal within the initial phase of the measurement. These fluctuations are mainly a result of sedimentation processes within the measuring cell and/or the dissolution of charged compounds from the sample causing a too early and wrong surpassing of the measurement threshold resulting in a wrong detection time.

☞ If measurements have been started by accident with an activated drift compensation function, this can be corrected after the measurement has been finished during data evaluation (see section 5.2). Thus the signal is reverted into its original shape.

3.4.6.2.2 "DropStop"

If this function has been activated in the "measurement parameters" window (fig. 3.7), then the measurement signal is not allowed to drop. Any decrease is transformed into a horizontal signal. Using this, negative drifts of the measuring signal/curve could be avoided or corrected.

☞ If measurements have been started by accident with an activated "DropStop" function, this can be corrected after the measurement has been finished during data evaluation (see section 5.2). Thus the signal is reverted into its original shape.

3.4.7 Storing and selecting measurement methods

With the exception of the incubator temperature (can be saved as information only), all parameters outlined in sections 3.4.2-3.4.6 can be stored in memory. They are then easily selected in a single step for subsequent measurements.

To save analyses select "Edit" from the "Analysis" menu. The "Edit analyses" window will appear and analysis parameters to be stored can be entered into the table.

A new set of fields for entering analysis parameters will appear after the **"+"**button has been clicked. New settings need to be confirmed by clicking the **"✓"** button. The **"x"** button will discard non-confirmed settings, and the **"-"** button can be used to remove the analysis highlighted.

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The buttons “ ▸ ”, “ ◂ ”, “ ▴ ”, “⏮” and “⏭”are used to navigate within analysis already stored.

When storing an analysis, a direct link to an previously stored calibration is possible. To establish that link the precise name of the calibration must be entered within “CalName” while entering the parameter to be stored for the current analysis.

Stored analyses can be selected in the “Analysis” field of the “measurement parameters” window. The selection has to be confirmed by clicking the “🡻” button beside the analysis selection field.

Alternatively the parameters set for an individual measurement position can be copied by using the “Copy all field contents” function (= shortcut “Alt+C”) in the “Edit” menu of the “measurement parameters” window and can be entered for other positions by selecting the “Insert all field contents” function (= shortcut “Alt+V”).

- ☞ Parameter settings that have been changed appear in a red font and need to be confirmed by clicking the red confirmation button (fig. 3.7). Then the modified parameters are definitely entered for the selected position and displayed in standard black colour. Confirmation is possible after entering the parameters in one field or after total parameter setting for one position is complete. As an alternative, parameters that have been changed can also be confirmed by pressing the “Enter” key for individual changes. Using the shortcut “Alt+Enter” confirms all settings that have been changed (same function as the confirm button).

- ☞ By clicking the “recycle bin” button all settings that have not been confirmed (red text colour) will be discarded, resulting in a reset to the previous parameter settings.

- ☞ For proper application of calibrations they should always be linked to an analysis.

3.4.8 User identification

To save the measurement parameters of a position the respective user entry in the pull-down menu of the field “User ID” must be selected (fig. 3.7).

- ☞ Users can be managed by selecting “User administration” from the “Extras” menu of the BacMonitor overview window.

- ☞ Every change of data requires (re-)identification of the user.

- ☞ The ID of a user who entered (and therefore possibly changed) one or more parameters recently is recorded in the database.

3.4.9 Extended copy functions for measurement parameter alignment

Extended copy functions for the simultaneous alignment of measurement parameters and sample ID to various positions within one incubator or for copying all parameters of one

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incubator to another are available when selecting the function “Edit” within the BacMonitor overview window.

Explanation of the functions available:

- **“Copy position”**
  Using this function the measurement parameters (analysis, evaluation, calibration, thresholds, time limits, etc.) and sample ID of a position focused via selection with the mouse will be copied to the data clipboard. The copied position is marked with a red dotted circle.
  - ☞ This function is only applicable to those positions which have been edited and saved by a user at a time before a (new) measurement is started.
  - ☞ This function is also accessible from a context menu which appears after moving the mouse pointer over the respective position and clicking the right mouse button.

- **Selection of target positions**
  After a position has been copied, the target position(s) can be selected individually. By clicking the left mouse button while simultaneously pressing and holding the “Ctrl” or “Shift” key, more than one position can be selected. They will be shown with a purple circle aligned.

- **“Insert sample ID”**
  Using this function the sample ID of a position copied to the data clipboard will be inserted into the selected target position(s).
  - ☞ This function is also accessible from a context menu which appears after moving the mouse pointer over the respective position and clicking the right mouse button.

- **“Insert parameters and sample ID”**
  Using this function the measurement parameters (analysis, evaluation, calibration, thresholds, time limits, etc.) and sample ID of a position copied to the data clipboard will be inserted into the selected target position(s).
  - ☞ This function is only applicable to those target positions which have not started a measurement yet. For target positions with ongoing measurement a respective error message will appear and the data of the data clipboard will not be applied.
  - ☞ This function is also accessible from a context menu which appears after moving the mouse pointer over the respective position and clicking the right mouse button.

- **“Copy incubator”**
  Using this function the measurement parameters (analysis, evaluation, calibration, thresholds, time limits, etc.) and sample ID of all positions of an incubator will be copied to the data clipboard. The copied positions are marked each with a red dotted circle aligned.
  - ☞ This function is only applicable to those positions which have been edited and saved by a user at a time before a (new) measurement is started.

- **“Insert sample ID into total incubator”**
  Using this function the sample ID of all positions of an incubator copied to the data clipboard will be inserted into the equally numbered positions of another incubator. To get the target selection done before, any position of the target incubator can be focused.
  - ☞ This function is only applicable to those target incubators which are of the same type of the source incubator.

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- **“Insert parameters and sample ID into total incubator”**
  Using this function the measurement parameters (analysis, evaluation, calibration, thresholds, time limits, etc.) and sample ID of all positions of an incubator copied to the data clipboard will be inserted into the equally numbered positions of another incubator. To get the target selection done before, any position of the target incubator can be focused.
  - ☞ This function is only applicable to those target incubators which are of the same type of the source incubator.
  - ☞ This function is only applicable to those target positions which have not started a measurement yet. For target positions with ongoing measurement a respective error message will appear and the data of the data clipboard will not be applied.

- **“Clear data clipboard”**
  This function clears the data clipboard. The red dotted circles aligned to the copied positions will be removed.

- **“Sample ID edit table”**
  Using this function a window for simplified entering of sample ID for the focused incubator will be opened. Choosing and copying within the table can be performed with the shortcuts “Ctrl+C” resp. “Ctrl+V”.
  - ☞ Every change of data requires (re-)identification of the user.

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4 Performing tests

The measurement parameters (see section 3) can be set immediately before inserting prepared measuring cells for measurement, before the end of the warm-up period (for already inserted measuring cells) or before the sample preparation.

It makes most sense, however, to set parameters for new investigation routines before sample preparation.

The selection of pre-set measurement routines is possible, however, immediately before inserting the measuring cells or afterwards, before the end of the warm-up period.

4.1 Preparation of measuring cells for the direct impedance method

The sterile measuring cells (glass or polystyrene) are filled immediately before use with the corresponding prepared and sterilised impedance media under antiseptic conditions (sterile work bench), or taken from a container that has been previously prepared and stored refrigerated (2-8 °C).

For measuring cells that are to be used for quantitative investigations, care must be taken to observe strict constancy of the pre-determined volumes (usually 9.0 ml), since variations in volumes can result in altered impedance signals.

We recommend the use of suitably precise pipettes or dispensing equipment.

The measuring cells have volume marking on the sides for use in purely qualitative analyses.

No further preparation is necessary when using pre-filled measuring cells (supplied complete).

☞ Measuring cells which have been stored in refrigerated conditions must be brought to at least room temperature before use. For this purpose they can be placed in empty incubator positions if not in contact with the electrodes (insert at 90 ° to normal position).

4.2 Preparation of measuring cells for the indirect impedance method

The polystyrene measuring cells for the indirect impedance method can be used up to five times. It is therefore useful to mark the number of analyses carried out on each cell. The tops of the measuring cells, however, can only be re-used when additional gaskets are used.

Immediately before use, the necessary measuring cells are each filled with 1 ml of a freshly prepared (as far as possible) 0.2 % KOH solution (0.2 g KOH chips on 100 ml A.dest.).

☞ The KOH solution can be stored, sealed, for up to a week at 2-8 °C. Ensure, however, that the volume of air above the solution is kept to a minimum. A KOH solution which has been stored for a longer period can be regenerated by boiling up for a short period onetime, but make sure that the concentration does not alter due to water evaporation.

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In the indirect method, nutrient and sample solution are transferred into the *inner measuring vial*. This is a disposable polythene container that has been supplied already sterilised via radiation.

Filling of the polythene vials with sterile nutrient should take place under aseptic conditions (sterile workbench).

Standard volumes used for the indirect method are 5 ml nutrient and 1 ml sample solution (sample homogenate). If a larger sample volume is to be investigated the concentration of the nutrient medium must be adjusted accordingly (e.g. 3 ml sample solution and 3 ml double concentrated nutrient medium).

Inner vials filled with sterile nutrient medium can be stored at 2-8 °C depending upon the shelf life of the nutrient solutions (see instructions for use). In this case, the vials need to be adjusted to the ambient temperature before use.

4.3 Sample preparation and inoculation of measuring cells

4.3.1 Liquid samples

Liquid samples require no special sample preparation. They are mixed accordingly and used for the investigation without further dilution.

1 ml sample solution is transferred into a measuring cell filled with 9 ml nutrient using a micro-pipette with a sterile tip. Shake the measuring cell gently. The measuring cell is placed into an empty measurement position of the *BacTrac* incubator immediately after addition of the sample.

☞ For all investigations using the direct impedance method with polystyrene measuring cells, after placing the samples in the incubator (or alternatively directly beforehand), turn the lids approx. 1/4 to 1/2 a turn anticlockwise to allow an adequate oxygen supply during incubation.

The time difference between inoculation and placing the measuring cell in the incubator should not exceed 15 minutes.

The theoretical detection limit for the investigation of liquid samples is >1 CFU/ml.

4.3.1.1 pH value correction

If sample homogenates whose pH value differs significantly from the pH value of the nutrient medium being used (e.g. sour milk), the pH value is neutralised by adding 1 N NaOH in corresponding quantities. The required quantity is to be determined in a trial and can be assumed when producing the nutrient medium for all future analyses of the same sort.

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4.3.1.2 Preserved samples

If a product contains an inhibitor (e.g. a preservative) we recommend diluting that product for impedance analysis purposes. To the diluted sample should be added a suitable inactivator for the inhibitor. In addition, the impedance medium can and should be used with an inactivator (e.g. *Supplement-I*). Proceed as described in 4.3.1.

4.3.1.3 Sample filtration

To increase the detection reliability or to investigate larger sample quantities (sample volumes), liquid and filterable samples can also be filtered via conventional filtration systems. The filter is then introduced into the measuring cells filled with corresponding nutrient media for investigation.

In this case the filter should be folded and then introduced into the measuring cells using sterile tweezers. Ensure also that the whole filter is covered with the nutrient medium. Contact between the filter and the electrodes has no effect on the measurement signal.

4.3.2 Solid samples

Solid samples must also be homogenised before investigation. Homogenisation takes place using stomachers as well as for the conventional microbiological investigation.

In this process each 10 g sample is typically homogenised with 90 ml of a sterile ringer solution or sterile peptic water. For the *BacTrac* investigation, 1 ml of this homogenous solution is dropped into 9 ml nutrient medium in the pre-prepared measuring cells as described for liquid samples (see section 4.3.1).

If necessary, correct the pH value of solid samples using a suitable buffer (e.g. buffered peptone water) when the sample is being prepared.

The theoretical detection threshold for the investigation of solid samples is > 10 CFU/g.

4.3.3 Swab samples

Sterile swabs (e.g. cotton wool swabs by Greiner) can be used to sample surfaces and/or difficult to access equipment parts.

To take a sample from dry surfaces the swab should be moistened before use with sterile ringer solution. To take a sample from a damp surface, apply the swab directly to the selected area.

Avoid touching the swab near its end since it could become contaminated by skin bacteria.

After taking a sample (swab) from a surface the head of the swab is cut off using scissors whose blades have been dipped in 90 % alcohol and heat sterilised in the flame of a Bunsen burner so that it falls straight into the measuring cell containing nutrient medium.

If investigating disinfected surfaces, add *Supplement-I* or another suitable deactivating material to the nutrient medium.

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Touching the electrodes with the swab has no effect on the measurement signal.

4.3.4 Specific information for the use of indirect measuring cells

In contrast to direct measuring cells, indirect measuring cells must be tightly closed. It is necessary in this case to re-tighten the lids of the measuring cells which have already been tightly closed immediately before placing the cells into the *BacTrac* incubator as the sealing lip of the lid loosens slightly within the first 10 minutes after initial closing the cells. For this reason it is also necessary to use a new lid for each measuring process.

For the indirect impedance method, however, inner vials must not be tightly closed, otherwise the CO_2 cannot escape. The polythene inner vial is therefore placed into the measuring cell with an open flip cap in order to allow the carbon dioxide (CO_2) to escape.

☞ Although the measuring cells can be used several times for the indirect method, the KOH solution must be made fresh each time.

For cleaning of the indirect measurement cells for re-usage only deionised water should be used.

It is necessary to completely dry the measuring cells before re-using them. For drying a maximum temperature of 50 °C should not be exceeded.

☞ A dishwasher is not mandatory for cleaning of indirect polystyrol measuring cells.

4.4 Insertion of measurement cells into the incubator

After the measurement cells have been inoculated with the test sample, they should be inserted into an empty position of a *BacTrac* incubator without any delay.

They will fit very easily into the respective position by twisting it slightly. After the measurement cells have been inserted they will be recognized by the system automatically during the next measurement cycle and the program for the respective position will be started according to the pre-set parameters.

☞ The sample number (dataset number) will be aligned automatically by the system after the warm-up time has expired and the measurement will start. Until that time the sample number still remains at “0”.

4.5 Finishing of measurements

Regularly measurements are finished automatically after the pre-set measurement time has expired. If the measuring cells are then not immediately removed from the incubator, the system still will continue with the measurement but the results are not registered in the curve display. Only if the cells are removed or the “Stop” function is activated, registration of measurement data for the respective positions will stop.

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It is therefore possible to prolong the pre-set measurement times as long as the cells are still in the incubator even if the pre-set time is already expired.

If measurement cells have been taken out of the incubator, the position will be marked “empty” during the next measurement cycle and is again available for a new measurement.

- ☞ After a measurement has been finished and the position has been marked “empty” again, the sample ID will be reset. The measurement parameters are preserved.

- ☞ It is possible to remove measuring cells between two measurement cycles but they must be inserted again before the next measurement cycle is active for the corresponding position. It is important to keep the temperatures of removed measuring cells relatively constant around the selected incubation temperature because otherwise signal drifts may occur.

The instruments are manufactured for permanent use and it is therefore not necessary to wait until the longest lasting measurement within an individual incubator has been finished to stop the measurement mode for a total incubator to finish measurements.

The recommended way to finish a measurement is just to remove the respective measurement cells.

If a typical result has been obtained before the set measurement time has expired, the cell can be removed to perform new measurements on the same position.

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5 Display and evaluation of measurement results

5.1 Display options and evaluation of measurement results using the BacTrac measurement software (BacMonitor)

Use the function key F6 or double-click with the mouse key slightly outside a circle icon within the BacMonitor overview window to access the "BacMonitor measurement parameters" window. Use the function key F7 or double-click within the circle icon in order to access the BacMonitor measurement window for the curve selected and the neighbouring curves.

To revert to the BacMonitor overview window, close the application window selected or use the function key F5.

5.1.1 BacMonitor overview window

The overview shows the following information:

- Measurement duration/end of measurement.
- Sample ID (field “Info1”).
- Sample status (evaluation).

5.1.1.1 Measurement duration

As a measurement cycle begins, a blue ring is superimposed onto the circle diagram whose circumference corresponds to the total duration of the warm-up phase plus the measurement period (pre-set in the "measurement parameters" window).

The duration of warm-up and measurement periods are indicated using different colours for each stage; **light blue (duration of warm-up phase)** and **dark blue (actual measurement duration)**.

As the measurement period for each position progresses, the circle segments turn dark blue, indicating the extent of progress.

At the moment of detection (measurement signal exceeds the set threshold value) the blue colour changes to indicate to the user the approximate impedance detection time.

5.1.1.2 Sample status (evaluation)

If the traffic light evaluation system is in use (red/green or red/yellow/green, see section 3.4.5.1), the ring around the circle icon in the overview window is also red/green or red/yellow/green, corresponding to the pre-set evaluation criteria and the pre-set time in relation to the total measurement period.

The warm-up phase is still indicated by light blue.

The progress of the measurement is still indicated by the dark blue-filled circle segments.

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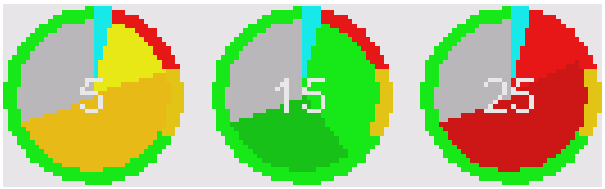
If the measurement signal exceeds the pre-set threshold value, however, the entire part of the circle is immediately displayed in the colour corresponding to the sample evaluation (red or yellow).

In addition, from the moment of detection, the relevant evaluation colour changes and the analysis continues in this new colour for the remaining duration (fig. 5.1).

This makes it easy to determine whether detection took place quite early in the cycle or towards the end of the period in the case of a red evaluation.

- ☞ If no detection is made before the transition time for red to green or for yellow to green, the blue-filled portion of the circle changes to green.
- ☞ If detection takes place after this transition time, the green is also correspondingly colour-graduated.

Fig. 5.1: Examples for sample evaluation



The end of the measurement is indicated by stating the time (for a measurement duration of less than 24 hours) or the date (for a measurement duration lasting longer than 24 hours) in a text window opened as soon as the mouse pointer is moved over a circle of an ongoing measurement.

5.1.1.3 Sample ID

The sample ID displays the contents of the first information field from the “measurement parameters” window. The text appears below the circle diagram as described above and is abbreviated as required.

5.1.2 Individual view (measurement curve)

The “measurement curves” window displays a detailed view of the measurement curves based on the evaluation criteria for that position, as specified in the “measurement parameters” window.

Figure 5.2 shows a typical view of the measurement progress.

According to the selected sample evaluation method, the selected threshold value is displayed for the corresponding period in the colours red/green or red/yellow/green.

The parameters necessary for the evaluation, such as “Detection time M”, “Detection time E” and “Status” (= sample status) when using the traffic light system, are displayed along with the actual measurement curve.

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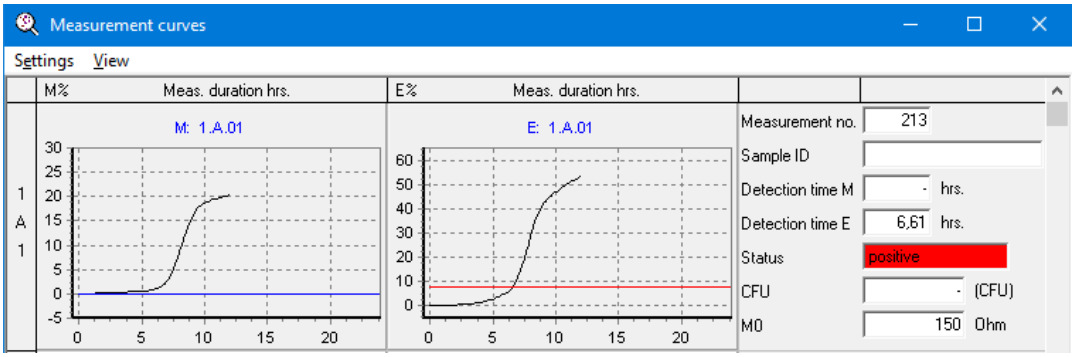
The microbiological count (“CFU”) corresponding to the detection time at the threshold set is only calculated and displayed if a calibration was selected for that measurement.

Fig. 5.2: Measurement process



Automatic evaluation of the samples takes place as described under 3.4.5.

- **Scale**
  The scale of the diagram can be adapted as required under "Settings" in the "Diagram axis" menu.

- **Display of measurement points**
  Individual measurement points can be displayed by selecting "Measurement points" in the "View" menu.

- **Zoom**
  The zoom function is activated by holding the left-hand mouse button and simultaneously drawing the mouse to the right, then releasing it.
  If you draw the mouse to the left, while holding down the left-hand mouse button then releasing it, the diagram will return to its original size (“unzoom”).

- **Quantity of diagrams and diagram size displayed**
  The quantity of the diagrams displayed is dependent on the configured resolution of your screen. Using the mouse pointer with the left mouse button activated on the left side of the diagram (below or above the position description) you can pull the line between two diagrams upwards or downwards. The diagrams can be adjusted to the window size by choosing the function “View/Adjust to window size”.

- **Browse**
  You can browse between the current measurement positions using the arrow keys “↑” (to browse upwards) or “↓” (to browse downwards).
  You can also browse page by page using the keys “Page ↑” and “Page ↓”.

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5.2 Correction of measurement signals

Corrections can be performed for ongoing measurements as long as they are still active. For already finished measurements this can be done using the “BacEval plus” software just to view and/or print these changes. None of the changes are saved to the database.

The "measurement parameters" window has, as already described in section 3.4.6.2, the option of correcting atypical measurement curves via the use of the “Drift compensation” and “DropStop” functions. Corrections have to be performed for M- and E-value individually.

5.2.1 Drift compensation

Caused by the sample material interfering with the conductivity of the nutrient, this can sometimes trigger signal jumps in the initial measurement phase. This results in the signal erroneously exceeding the pre-set threshold value and is followed by “Detection” and calculation of microbiological counts using calibrations.

Since detection on the basis of the above phenomenon is incorrect, it is possible to delete the erroneous part of the curve within a pre-determined time period and reset the remaining curve to the baseline.

☞ Set the time for correction of the measurement signal to the absolute minimum.

This results in the recalculation of the detection time and of the microbiological count (if a calibration has been used).

☞ Unusually early detection or unusually high microbiological counts can sometimes be explained by signal jumps at the start of the measurement cycle and can be corrected using drift compensation.

5.2.2 “DropStop”

In contrast to drift compensation, “DropStop” prevents the signal from drifting downwards. The function cancels out any part of the curve with a negative slope substituting it by a horizontal line. This allows correction of negative drift, i.e. a slide of the base-line of the measurement signal into the negative range.

This action cancels out the effect of negative drifts of the baseline which can significantly shift IDT or even prevent a typical microbiological signal from detecting at all.

☞ The “DropStop” function can have a significant effect on detection times and microbiological counts in case of strong negative drifts. In case of a product which is known to cause this kind of drift, the function can be activated by default in the corresponding analysis.

5.3 Data evaluation with BacEval plus

A more detailed evaluation of measurement results, not only based on the simple options for monitoring measurement results using the *BacTrac* measurement software (BacMonitor) is

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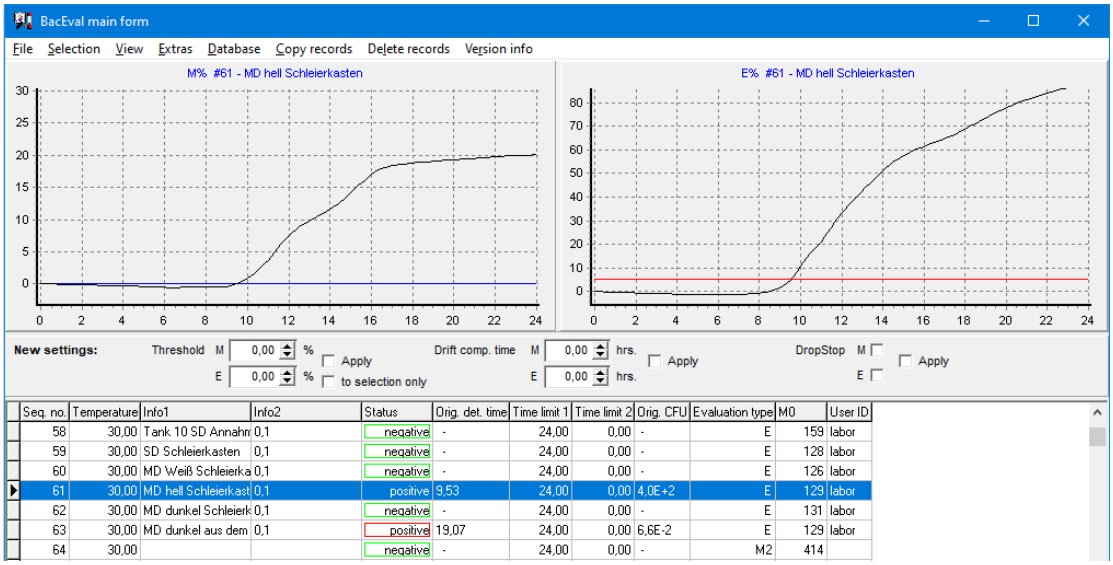
carried out using the “BacEval plus” application which can also be found in the program group *“BacWin2”*.

5.3.1 Selecting measurement results

After opening the “BacEval plus” application you will access the application overview window (fig. 5.3).

Fig. 5.3: “BacEval plus” main form



☞ You can set the language within the "Extras" menu as described for BacMonitor (see section 3.1.8.1).

Before you can start with the evaluation of data you have to select it. Use the function "File/Select” to open the window in which you can select measurement results (fig. 5.4).

The following parameters can be used as search criteria:

- Incubator temperature.
- Sample ID and information entered in the Info fields.
- Sample status.
- Date of analysis (from/to).
- Instrument serial number.

All the criteria selected for the search for measurement results are linked using the "and" function. If you enter 37 °C for the temperature and SYL* in the information field 1 and select "positive" for the evaluation, then click the “Execute” button, the application carries out a search for those samples whose incubation temperature was 37 °C, whose sample ID starts with "SYL" and which are evaluated "positive".

For a text search within the info fields the following options are available:

- Full text:

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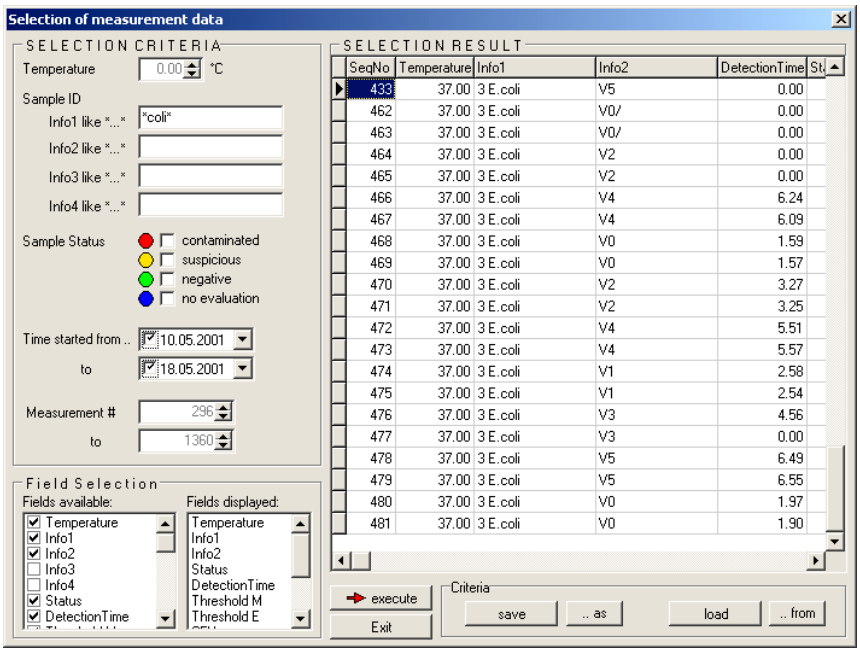
Exactly the text entered will be used for the search operation.

- If a search text is surrounded by * i.e. *coli*, the search will be performed for any word containing the term “coli” elsewhere.
- Search for initial letters:
  If texts will be entered in the way of *abc%*, the search will be performed for any words starting with “abc” and % stands for any letter.

Non-activated selection criteria are greyed out.

To customise your results table use the "Field selection" function to select from the "Fields available" list in the results display for each field. These are then transferred to the "Fields displayed" window. Doing this, only the fields you want to display will be shown in your results table, all other (not selected) information is suppressed.

Fig. 5.4: Selection window for measurement results in “BacEval plus” application



In addition, the priority for sorting the selected fields can be defined in the window “Sort order”.

The customised selection criteria can be stored on demand in the application directory *“BacWin”* as files with the extension “.cnd” using the buttons “Save” or “.. as”, and “Load” or “.. from” to reload them. If the function “Save” is used, the configuration will be saved in a file named “Default.cnd”, otherwise the created file can be named by the operator.

After entering your search criteria and selecting the fields to be displayed, start the search by clicking the “Execute” button. The table “SELECTION RESULT” serves informational purposes only.

After the measurement results have been selected, the window is closed to return to the main form window by clicking the “Exit” button.

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5.3.2 Display of measurement results

The selected data records are integrated immediately after activation of the “Execute” button into the “BacEval plus” main window (fig. 5.3) and can be displayed as any combination of individual diagrams and/or total diagrams and value tables. If the function “Highlight selection in total diagram” is activated, the measuring curve of the respective diagram selected will be highlighted by drawing it in a thicker line.

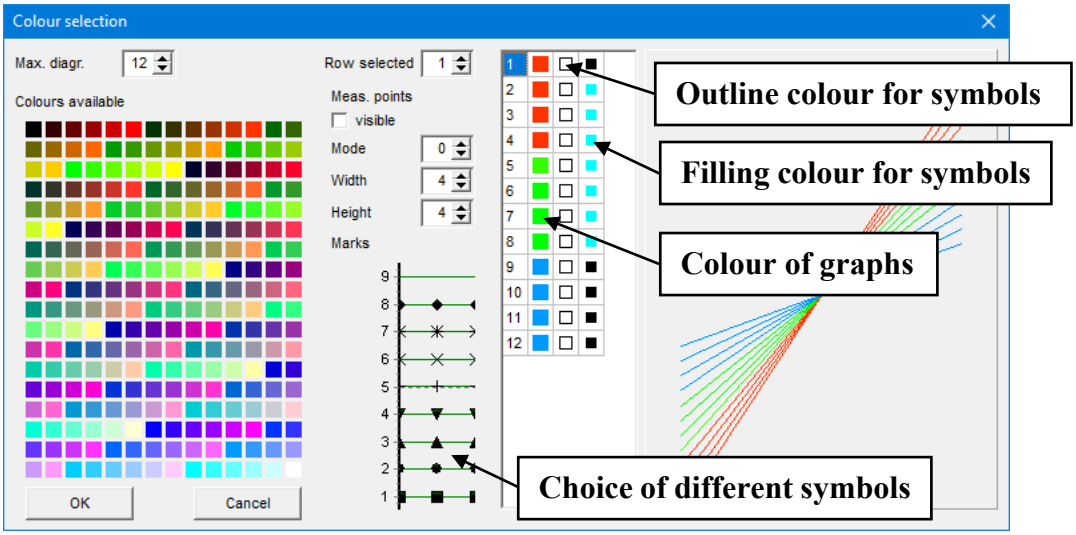
The display mode for the various diagram types is selected using the “View” menu. Both a single diagram and total diagram exist for the M- and the E-value. The single diagram displays only a single graph, while the total diagram can show several graphs in one diagram.

In the table view you can browse within a selected diagram using the mouse or the up/down arrow keys. The corresponding single diagrams will be shown simultaneously in the left half of the window. Individual diagrams selected in this way can be integrated into a total diagram display window (right half) using the "Enter" key.

The respective size of both diagrams can be adjusted. Place the mouse cursor exactly between the diagrams shown. A left/right arrow symbol appears. Click and hold the left mouse button and move the pointer towards the diagram you want to shrink, then lift the button again.

For the differentiation of measurement curves within the total diagram window the colours must be selected and aligned using the ”Colour selection” function within the “Extras/Diagrams” menu (fig. 5.5).

Fig. 5.5: Window “Colour selection”



☞ Altered colour settings are defined as new defaults.

The selected colours selected will appear in the total diagram mode as well as a colour flag left of the measurement number in the display table for each selected result (fig. 5.6).

With the “Max. diagr.” setting the number of differently coloured graphs can be set somewhere between 8 and 30. New colours can be selected by clicking on a box on the right side, then

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clicking on the preferred colour in the “Colours available” section. For each row three settings are available: The colour of the graph (= first column), the colour of the outline of the symbols (= second column) and the colour of the filling of the symbols (= third column).

In addition, nine different symbols for displaying the curves can be selected. For these symbols the width and height can also be altered.

The colours and/or modes selected are displayed as an example within the diagram on the right.

- ☞ A theoretical selection of 256 different colours is possible. For practical reasons an easy distinguishable colour alignment is only possible for 10 to 14 curves.

You can select a new threshold value for the selected data record by selecting “New threshold”. The detection times and CFUs (if a calibration has been used) are recalculated automatically and are shown in red font. The new detection times for M and E are displayed within the “New det. time M” and “New det. time E” column.

- ☞ The calculation of new detection times for changed threshold values takes place only in the table display for the evaluation and has no effect on the original detection times stored in the database.

- ☞ The detection time displayed within the column named “detection time” represents the detection time that has been registered during the original measurement and which is stored in the database. It is directly linked to the type of evaluation performed and can be therefore a detection time for M or for E. If no evaluation has been selected for the measurement no detection time is stored in the database and the value remains “0”. During recalculation of thresholds it will be changed and marked red if the original detection threshold has been changed. This change is not permanent and not saved to the original stored value.

- ☞ The two columns “New det. time M” and “New det. time E” together with “Orig. det. Time” and “Orig. CFU” are part of the default selection everytime BacEval plus is started (see section 5.3.1).

Figure 5.6 shows a typical example for a combined display of single diagram, total diagram and result table. The curve presented in the single diagram window is highlighted in the total diagram and the colour flag appears also within the result table.

Also the sample status (red/yellow/green/blue) is displayed as a coloured frame around the status column within the result table.

Using “drag and drop” the different columns within the result table can be re-arranged if necessary or wanted.

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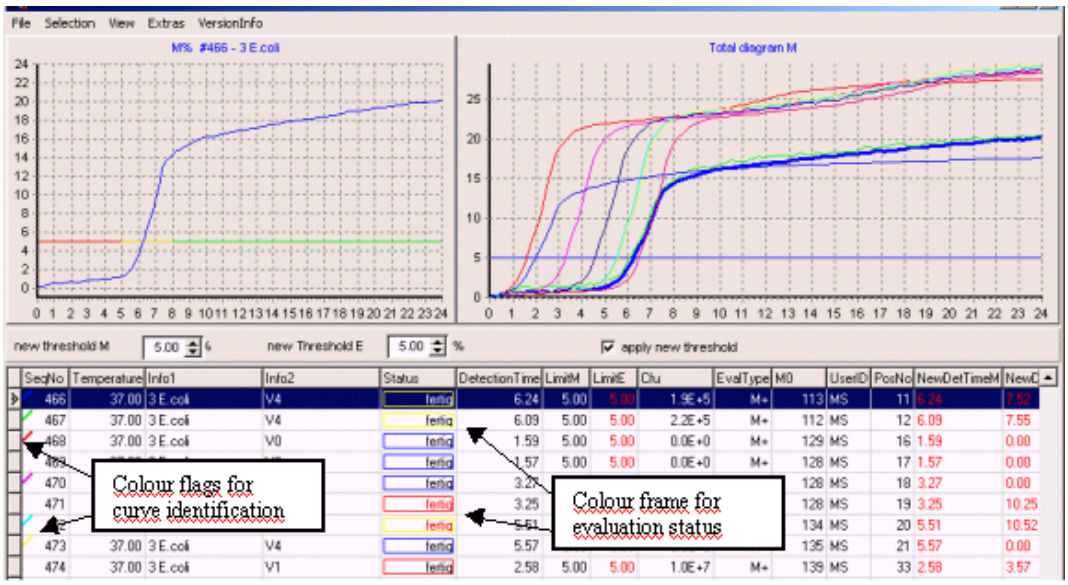
Fig. 5.6: Display of measurement results



5.3.3 Data printouts

Selected measurements can be printed using the "Print" function within the “File” menu using the following formats:

- Individual diagrams.
- Total diagrams.
- Table.

☞ The use of a colour printer is recommended for curve identification within the total diagram.

☞ For printing on non-colour printers the “Print black/white only” checkbox must be activated.

5.3.4 Exporting data

The “Export selection as table” function found in menu “File” is used to export the contents of the result table as a text file with columns separated by tab characters to a directory specified by the user. The tabulated results can then be imported into any supported data processing application (e.g. MS Word, MS Excel, etc.) and processed further.

The “Export selection as single files” function in menu “File” is used to export the measurement data of selected curves as several text files with columns separated by tab characters to a directory specified by the user. The tabulated results can then be imported into data processing applications and processed further.

Statistical evaluations are therefore very easy to perform, e.g. using MS Excel statistical functions.

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Furthermore selected data can be exported by the function "File"/"Export selected measurements to database" and imported into an existing database of a different PC system by choosing "Import measurement(s) into database".

5.3.5 Opening and closing of a secondary database

Alongside the primary database it is possible to open an additional (secondary) one with "BacEval plus". It can be in the old format (up to *BacWin* 1.07) or in the new format (starting from *BacWin* 2.00).

The database that is currently activated (= processed) is displayed in the status bar of "BacEval plus".

The primary database (= operating database) always remains open. It is possible to switch between the two open databases.

☞ Only one additional database can be open at the same time besides the primary database. If a secondary database is opened it is not possible to connect to another one until the secondary is closed again.

5.3.5.1 Opening a secondary database (old format)

This can be done with the function "File/Open BacTrac database (old format)". A standard "Open" dialogue appears to let you define a directory in which the (old) database is stored. The database is opened by selecting the "Measurement.DB" file.

5.3.5.2 Opening a secondary database (new format)

This can be done with the function "File/Open BacTrac database (new format)". A window appears in which the list on the right displays all ODBC databases available on your system.

Proceed as follows to open a link for the first time:

1. Select database from the list on the right. The right button "Connect" becomes accessible.
2. Enter database login information. If you have not changed the user configuration of your Firebird installation (yet), enter the username of the database administrator **"SYSDBA"** and the password **"SyFbBMX!"**. (*BacWin* 2.60 and older versions have not changed this password while installation, therefore it can also possibly be **"masterkey"**.) (If your login fails, either the credentials of the database administrator **"SYSDBA"** have been changed or this account is disabled. In these cases please contact the person who is responsible for the security of your Firebird installation(s) at your corporation.)
3. Click "Connect" button.

When the link is established the database is included in the list on the left and the username and the password are saved permanently. Now this database can be selected from that list. The "Connect" button below the list becomes accessible. Clicking the button opens the database and closes the window.

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5.3.5.3 Switch between both databases

The menu “Database” allows you to switch between both databases.

5.3.5.4 Closing the secondary database

Select the secondary database as active database. It can be closed now by selecting “File/Close current database”.

5.3.6 Creation and deletion of secondary databases

By selecting the “BacEval_plus” menu “Extras/Create or delete database” a secondary database can be created. The procedure is identical to the creation of databases with “CreateDB” described in chapter 3.1.3.

5.3.7 Temperature log (BacTrac 4310)

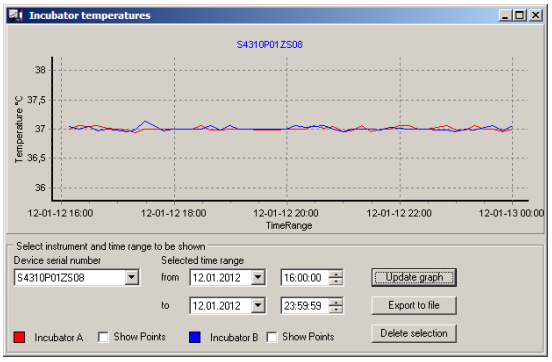
The temperature data will be automatically logged during operation. This data can be accessed via the “BacEval plus” function “Temperature log” from the “View” menu.

To display data select the serial number of the instrument, enter the time range and click the button “Update graph”. The diagram displays measuring data from both temperature sensors. There is also an option for export or deletion of data.

☞ Temperature data of *BacTrac* 4300 instruments and older models of the 4000 series (4100 and 4110) will not be logged.

☞

Fig 5.7: Temperature log



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6 Calibration and setting evaluation criteria

The temporal development of the measuring signal serves as a measure of the microbial load of the examined sample. The earlier the pre-set threshold is reached, the higher the sample is contaminated with microorganisms, since the change in impedance occurs more rapidly the more microorganisms are present in the sample.

The impedance detection time responds indirectly proportional to the germ count in the sample.

Therefore higher germ counts give shorter detection times and lower germ counts give longer detection times. This has the great advantage of establishing exceeded guidance levels early on.

The proportionality between the germ count of a sample and the impedance detection time is usually linear over a very wide range when the impedance detection time (x-axis) is plotted against the decimal logarithm of the germ count (y-axis) determined by reference methods in an x-y diagram (10^3-10^7 CFU/g resp. ml).

This relationship can be determined mathematically in most cases by means of **linear regression.** More complex dependencies can also be determined by means of quadratic or cubic regression.

The most essential criterion for establishing of a calibration, however, is the absolute necessity for parallel investigation of genuinely identical samples by means of impedance and reference processes. As the same sample preparation is usually used in both methods, this is actually quite simple.

6.1 Requirements for and influential values in establishing a calibration

When determining a germ count using the plate method the resulting visible colonies are counted after a pre-set incubation period and it is never known whether a visible colony has germinated from a single bacterium, or whether it is the product of several bacteria (e.g. streptococci, staphylococci).

On the other hand with impedance measurement each individual bacterium contributes proportionally to the generation of the impedance signal. The impedance measurement is thereby a dynamic process in contrast to the plate method which is an end point process. It is influenced by the following parameters:

- Sample matrix.
- Bacteria spectrum.
- Metabolic situation of bacteria.
- Incubation temperature.
- Sample and medium volumes.

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6.1.1 Sample matrix

Both nutritive and inhibiting substances can be introduced in to the measuring cell via the sample matrix.

For the production of a calibration it should be ensured that mainly samples with an identical matrix are classified as far as possible. In principle it is possible to use different starting materials, but this requires special consideration during establishment of the regression curve.

If it turns out that values from a particular sample type do not comply with the values of another sample (different germ counts on the plate over similar impedance detection times) those samples should be calibrated separately.

6.1.2 Bacteria spectrum

The bacteria spectrum present in the sample is of enormous significance to the calibration process.

As metabolism varies for different bacteria species and there is therefore a possible influence on the signal generation in the impedance analysis, as far as possible, only products with similar bacteria spectra should be classified together for the calibration.

Milk is a good example. While both raw and pasteurised milk hardly vary in sample matrix, the bacteria spectra differ greatly. While predominantly gram negative bacteria are to be found in raw milk, pasteurisation initiates a selection process in favour of gram positive bacteria. As gram positive and gram negative bacteria have different signal behaviours, these products must be separated for calibration processes in order to increase the calibration predictability.

6.1.3 Metabolic situation of bacteria

Bacterial contaminations in dry products have a completely different metabolic situation compared to the same bacteria present in a liquid product or in a product with a high water activity (a_w value).

Therfore a product with low water content requires a longer adaptation phase for proliferation of bacteria in nutrient media during the biological analysis.

Impedance analysis is a dynamic process in which time plays a significantly greater role than in the plate process. This factor should therefore be taken into consideration accordingly. For the production of a calibration, only products with similar a_w values should be classified together.

Let us take the investigation of milk powder and pasteurised milk as an example. The products do not differ either in their composition or in the potentially occurring bacteria spectrum (predominantly gram positive bacteria) but the metabolic situation for possible contaminants is fundamentally different. Even in this case, therefore, separate classification is necessary for the calibration.

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6.1.4 Incubation temperature

The proliferation rate and the metabolism of microorganisms are fundamentally influenced by the incubation temperature.

For calibration purposes it is therefore essential that all investigations in the *BacTrac* as well as in the reference processes are carried out at the same temperatures.

Since thermally processed samples in impedance analysis give significantly more reproducible results at an incubation temperature of 37 ° C compared to 30 ° C (reference method), it is sometimes absolutely necessary for impedance and reference methods to be carried out at different temperatures.

It is important, however, that the temperature *per process* is kept the same. It is imperative that the results used for calibration have been obtained using the same incubation temperatures for that method.

6.1.5 Sample and media volumes

The change in impedance is also volume dependent. Although the relative measurement used in the *BacTrac* makes the process more robust in this respect, take care nevertheless to use constant volumes of nutrient media and samples.

Fluctuations in sample volumes in particular must not exceed ±5 %.

6.2 Quantity and selection of samples for calibration

For statistical reasons, at least 30 and ideally 50-80 pairs of values or more should be used for establishing a calibration.

Another essential criterion however is the distribution of samples over the total possible contamination range. The distribution of the germ counts should range as far as possible across at least four log steps (e.g. 100–1,000,000 = 10^2–10^6 CFU).

The better the distribution of the samples, the more rapid is the establishment of the calibration. The investigation of a large number of samples in a very similar contamination range is therefore of little value.

Especially production environments, the majority of samples have very similar germ counts. In such cases, the methods described below for increasing or decreasing the germ counts of products are recommended.

In no case may products be artificially contaminated with separately grown cultures of microorganisms for the purpose of increasing the germ count.

In general the entire calibration should be carried out using only naturally contaminated samples.

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6.2.1 Reducing the germ count for calibration

To obtain a lower germ count it is possible to produce a series of dilutions of highly contaminated samples.

Naturally, the first choice of diluting agent is a sterile product. If this is not possible, dilution can be carried out using a conventional diluting agent (ringer solution or peptone water). However, it must be ensured that the product itself can exert an influence on the growth of the microorganisms and that this influence must never be changed too much by dilution.

The influence of the product can be determined using a simple test:

The product is investigated once undiluted and again in two serial dilutions (e.g. 1:10 and 1:100). The differences in the detection times between the successive dilution steps are compared.

If the difference is no more than ±0.7 hours then there is no significant product influence and dilution is admissible for the production of the germ count.

The test to determine a possible product influence should always be carried out in duplicates.

6.2.2 Increasing the germ count for calibration

6.2.2.1 Spoilage

Initiating natural decomposition within the product can increase the germ count of fresh products.

It is essential, however, that spoilage takes place at normal storage conditions for the product. Forced decomposition, e.g. increasing the storage temperature of refrigerated products is to be avoided as it can cause the flora of e.g. a psychrophilic contamination flora to be overgrown by a mesophilic flora, with potentially different impedance behaviour.

6.2.2.2 Artificial contamination with adjustment phase

As described above (see section 6.2) artificial contamination using freshly grown microorganism isolates is to be avoided as this can severely distort the true picture of the microbial contamination.

If, however, there is no other alternative to increasing the germ count in a sample, it is possible to attempt artificial contamination with adaptation to the product. It is necessary in such cases, however, to work as far as possible with typical product isolates and not pure cultures from various culture collections.

After inoculation of the product, a period of at least 12-18 hours under normal conditions should be allowed for the contaminant to adjust to the product. Only then can this kind of artificially contaminated sample be used for calibration purposes.

Calibrations produced in this way require particularly careful monitoring (see section 6.5).

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6.3 Performing the calibration

The investigation of samples whose results will be used for calibration purposes is not essentially different from the investigation of routine samples.

The points listed below should, however, be taken into account.

6.3.1 Sample preparation

For most applications, sample preparation is identical.

The same sample homogenate should be used for performing reference and impedance processes on solid or paste products. In this case there is no need to analyse sample dilutions for the impedance process.

If it is necessary to prepare samples differently, then take care to ensure the homogeneity of starting materials. The process for the sample preparation is described in section 4.3.

6.3.2 Reference method

Basically all integrating processes (pour plates, drop- and spiral plates, Petrifilm) can be used for the reference process.

If available, however, standardised procedures should be used.

Essential for the precision and predictability of a calibration is that the reference process is genuinely precise and is carried out according to set rules and that the same types of starting materials are used.

6.3.3 Sequential timing of impedance and reference method

As the microorganisms start metabolic activity immediately after a sample has been introduced in the *BacTrac* measuring cell, the following procedure for parallel investigations using impedance and reference method is recommended:

- Sample homogenisation.
- Production of dilution for reference process.
- Inoculation of *BacTrac* measuring cells using sample homogenates.
- Placing measuring cells into the *BacTrac*.
- Inoculation of plates for the reference process.

☞ Ensure that the time between inoculation of the measuring cells and placing them into the *BacTrac* incubator is less than 15 minutes if possible.

6.3.4 Establishment and calculation of calibration curves

As an additional advantage of the impedance splitting method, calibration can be performed either on the M- or on the E-value.

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☞ As a principal recommendation, the calibration should be based on those measurement curves (M or E value) which yield the best fitting calibration parameters (see below).

All measurements carried out for the production of the calibration are selected in the "BacEval plus" application and exported using the "export files table" function afterwards.

It is recommended that the selected data is exported several times using different threshold values each time (e.g. 3, 4, 5, 6, and 7 %M, and 5, 6, 7-10 %E respectively) so that the calibration calculation can be carried out at the best-suited threshold value.

The calculation of the regression equation and the graphical display of the regression lines is carried out via the accompanying Excel spreadsheet with the filename "Calibration_en.xltx". If the exported file is opened in MS Excel, the sample ID and associated file numbers and impedance detection times (for each threshold) are inserted via the import function of MS Excel. The CFU values obtained in parallel via the reference method are then entered in the "CFU plate" column.

The calculation of the regression calibration and the graphical evaluation is carried out automatically.

This process is repeated for the different detection times for each threshold. In this case, however, it is only necessary to replace "IDT" fields using copy/paste commands.

6.4 Evaluation of calibration lines

As well as determining the equation for describing the calibration lines (linear regression) or the calibration curve (polynomial regression), important parameters, **correlation coefficient (r)** and **dispersion (s_{yx})** for evaluating the calibration are displayed in the Excel sheet.

The closer the correlation coefficient *r* is to the ideal value -1 (= maximum compatibility, all values lie on the lines/curve described by the calibration), the more precise is the relationship between the impedance method and the reference method.

A correlation coefficient close to the ideal value on its own is not sufficient though, to evaluate the quality of the calibration. The dispersion (s_{yx}) must also be taken into account.

The dispersion, s_{yx} (given in log units) should be at least <0.5 log units.

From a calibration result with a correlation coefficient of e.g. r = -0.91 and dispersion of s_{yx} = 0.4 it can be extrapolated that 91 % (= r x 100) of values of the lines described by the equation (or curve in the case of polynomial regression) lie within a distribution of ±0.4 log (= s_{yx}) units.

There are no distinct directives on acceptable upper values for a correlation coefficient for biological processes. Basically it may be assumed, however, that r should be ≤-0.85. The negative prefix comes from the indirect proportionality.

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The value of *r* may be below -0.85 in exceptional cases if accompanied by a correspondingly small s_{yx}. In these cases the calibration must be monitored very carefully (see section 6.5).

☞ Calibrations are applicable only to those ranges covered by values within the calibration curve.

6.5 Checking calibrations

6.5.1 Checking the predictability in comparison to the reference method

☞ The statistical processes listed below are merely an example of a variety of choices. Other suitable statistical methods are also valid for review.

6.5.1.1 Two-sided t-test for paired samples

At least 20 samples, which should as far as possible cover the entire calibration range, are investigated using the calibrated impedance process and the reference process.

Assuming that the results are normally distributed, the logarithm (log 10) of the germ count results is produced and the hypothesis that for comparable processes the mean based on the difference of two parallel results on a significance level $\alpha = 5\ \%$ is equal to 0 is monitored using the t-test.

For each pair of values the difference is produced and from it the arithmetical mean (d') and its standard deviation s_d : d'.

The test value t_{pr} is calculated using the following formula:

$$t_{pr} = \frac{d`}{sd}\sqrt{n}$$

The test value t_{pr} is compared with the critical value $t_{f,1-\alpha/2}$ ($f = n-1$, $\alpha = 0.05$) which can be read off a corresponding t-test table showing the t distribution for f, 0.975.

The processes are equivalent i.e. the calibration corresponds if the following precondition is fulfilled:

$$|t_{pr}| \leq t_{f,1-\alpha/2}$$

If this precondition is not fulfilled there is a significant difference of results obtained with the two methods.

6.5.1.2 Regression analysis

Statistically experienced users can check a calibration using a regression analysis.

Here, as mentioned above, at least 20 samples are analysed using both methods (impedance and reference method) and the results are displayed in an x-y diagram. The values obtained from

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the reference method (independent variable) are entered on the x axis, while the results of the impedance analysis (dependent variable) are entered on the y axis.

Using regression analysis the probability that the model y = x will be checked, i.e. the comparability of the methods, is statistically tested.

Please refer to specialist literature to find out more about performing regression analyses.

6.5.2 Periodic testing of calibrations

As significant seasonal changes of the bacterial flora, particularly in raw products, cannot be ruled out, it is recommended that the calibrations are checked at regular intervals (3-4 months) but at least twice a year.

In this case a minimum of six but ideally ten to 20 samples should be investigated along with the reference process. The results should not differ by more than ± 2 x s_{yx} of the regression equation. If a clear trend is visible then you should consider re-calibrating the impedance process.

The above statistical process can be used for a more precise check.

6.6 Working with calibrations

6.6.1 Saving and selecting calibrations in the BacTrac software

After a calibration has been produced using the corresponding Excel spreadsheet, the required parameters are present for the transformation of the impedance detection times (IDT) obtained into corresponding germ counts.

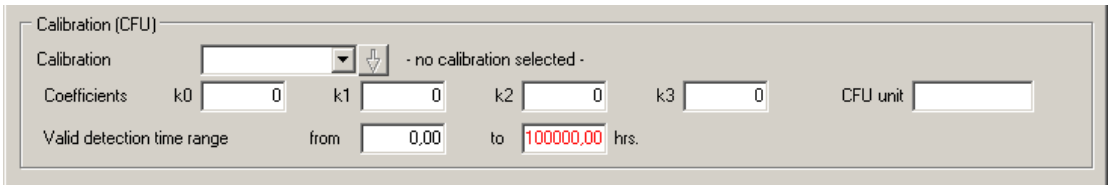
When using linear regression to calculate the calibration lines the mathematical relationship between IDT (*BacTrac*) and CFU (reference method) is described using the following equation type:

$$y\ (\log CFU) = \mathbf{k1}\ x + \mathbf{k0}$$

After entering both coefficients k0 and k1 in the field provided for the entry of calibration parameters in the “BacMonitor measurement parameter” window (fig. 6.1), the impedance detection times for the following measurement made in the corresponding measurement positions are transformed into CFU. At this time, the corresponding unit (CFU/g or CFU/ml) can be entered in the “CFU unit” input field.

For linear regressions, coefficients k2 and k3 are always 0.

Fig. 6.1: Range for the entry of coefficients in measurement parameters menu option



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For quadratic regression equations, the equation is extended by the quadratic term **k2 x²** and for cubic equations, additionally, by the cubic term **k3 x³**.

The values should then be taken into account again for the coefficients.

Examples:

1. The equation for a *linear regression* is as follows:
   y = -0.432 x + 7.43

   in which:
   k0 = 7.43
   k1 = -0.432

2. The equation for a *quadratic regression* is as follows:
   y = 0.021 x2 – 0.67 x + 13.72

   in which:
   k0 = 13.72
   k1 = -0.67
   k2 = 0.021

Additionally, from *BacWin* version 2.46 upwards, a valid time range for calibrations can be set: To do so lower and/or upper time limits can be entered (in hours) into the fields *from* and *to*. In this case all results outside of the set range will be displayed as "<" or ">" the corresponding value.

This is especially important for calibrations with quadratic equations, where the upper limit should be set equal to the time of the curve minimum. For linear regression, it is advisable to use the earliest and latest result contained in the calibration for lower and upper limit respectively. Using this option will restrict the validity of the calibration precisely to that range covered by real sample results. Extrapolation of values above or below areas not covered by the calibration is no longer possible.

To save a calibration select the menu option "Calibration/Edit" of the "BacMonitor measurement parameters" window. A table will appear and the calibration parameters to be stored can be entered there.

A new set of fields for entering calibration parameters will appear after clicking the **"+"** radio button. New settings need to be confirmed by clicking the **"✓"** button. The **"x"** button will discard non-confirmed settings and the **"-"** button can be used to remove the calibration highlighted.

The buttons **" ▶ "**, **" ◀ "**, **" ▲ "**, **"⏮"** and **"⏭"** are used to navigate within calibrations already stored.

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Stored calibrations can be selected in the “Calibration” field of the “measurement parameter” window. The selection has to be confirmed by clicking the **“🡻”** button beside the calibration selection field.

For the establishment of an automatic link for a stored calibration with an analysis see section 3.3.7.

- ☞ For using the calibration data correctly it is extremely important that the threshold (type and threshold value) which has been used for the calibration is considered.

- ☞ For a proper operation of the software a calibration should be linked directly to an analysis. (To link calibrations with analyses see section 3.4.7.)

6.6.2 Setting evaluation criteria (sample qualification)

6.6.2.1 Quantitative methods – three stage classification (red/yellow/green)

The criteria described in section 3.4.5 for automatic classification (red/yellow/green) of measurement results can be calculated using the calibration equation.

If the threshold, reference or warning values for a sample are known, they can be entered in the calibration equation to determine the time thresholds for the evaluation.

Example for calculating the evaluation criteria:

For product A the warning value is 5,000 CFU/g while the threshold value for the rejection of the product which cannot be exceeded under any circumstances is 50,000 CFU/g.

The following relationship between the germ count and the impedance detection time (t) was determined for the calibration equation for product A:

$$\log \text{CFU/g} = -0.432\ t + 7.267$$

Setting the warning value at 5000 and converting the equation in accordance with t gives the following equation for the subsequent calculation:

$$t = \frac{3.699 - 7.267}{-0.423} = 8.4 \text{ hours}$$

This means that all samples with a detection time of more than 8.4 hours fall below the warning threshold.

After inserting the known threshold value (50,000 CFU/g) in the equation above, a time value of t = 6.1 hours is obtained. All samples with a detection time below 6.1 hours thereby exceed the threshold value and must be rejected accordingly.

Classification criteria are summarised as follows:

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Sample CFU/g	Detection time (t)	Colour
>50,000	<6.1 hours	red (contaminated)
>5,000 <50,000	>6.1 <8.4 hours	yellow (suspicious)
<5,000	>8.4 hours	green (complies)

The classification times (see section 3.4.1) to be entered in the “measurement parameters” window must therefore be as follows:

Red: 6.1 hours.
Yellow: 8.4 hours.

☞ It is important for accurate evaluation of measurement results that the same threshold value for "Threshold M" is used as for setting up the calibration.

6.6.2.2 Quantitative method – two stage evaluation (red/green)

In the event that there is only one value at which samples are to be evaluated automatically, only this one value is to be entered in the calibration equation.

Only the time for **red** is entered in the "measurement parameters" window. This time is then automatically also taken over for the **yellow** classification.

For automatic classification this means, however, that differentiation is only made between **red** (threshold value reached or exceeded) and/or **green** (sample below threshold value).

6.6.2.3 Qualitative method – two stage evaluation (red/green)

Automatic sample evaluation can be used for investigations detecting purely presence/absence (e.g. sterility tests).

In this case, whenever the set threshold value M (%) is reached within the given measuring time, the result (sample) is classified as **red (= not sterile)** bacteria investigated is present. The total measurement time is therefore used as an input for the *red* classification in the “measurement parameters” window.

Any sample that exceeds the set M-threshold within the measurement period is evaluated **red**, and all those that have not reached this threshold by the end of the measurement period are evaluated **green**.

6.7 Distribution analysis for the evaluation of samples (alternative determination of evaluation criteria)

The distribution analysis can be used to appraise samples or products which fall outside the range required for calibration (4-5 log stages). This makes it possible to classify samples within the range above or below a specified threshold value.

The principle of the distribution analysis is that samples whose germ count is above a specified value are detected within a time span which is clearly separate from that time span within which the samples with acceptable germ counts are detected.

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On that basis two time limits are determined:

- **Time limit 1 (red)** represents the time which, if not exceeded, indicates that the germ count in a sample under investigation lies above a specified threshold value with high probability.

- **Time limit 2 (yellow)** defines the range in which the probability of the threshold value being exceeded is given, but within which a result just on or below the threshold value cannot be excluded (= uncertainty range or grey area).

All samples which do not exceed time limit 2 are classified as **green**, indicating a germ count (min. one log step) below the threshold value.

In contrast to establishing a calibration, only samples with germ counts above and approx. 1 log stage below the specified threshold value are required for the purposes of determining each time limit.

As described for the calibration, samples are investigated both via the impedance method and the reference method (see section 6.3).

The results are summarised in two tables. The first table contains only those results that lie above the specified threshold value. The second table contains all results with values below the threshold value.

Impedance detection times are given in hours. The number of samples detected within the given time phases are entered in the table along with their percentage proportion of the total number of results entered in the corresponding table (*n*).

These results are presented as a histogram (fig. 6.2) and the time limits are extrapolated from both tables.

- **Time limit 2 (yellow)** is the time within which all positive samples are detected and false negative results can be excluded (seven hours in example above).

- **Time limit 1 (red)** is the time within which the majority of positive samples (above the threshold limit) are detected (five hours in example above).

In this way the majority of samples can be reliably classified as falling above **(red)** and/or significantly below **(green)** the specified value. Samples which lie between limits 1 and 2 **(yellow)** are very close to the threshold and are counted as suspicious and should be repeated or confirmed with the reference method.

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Example:

Tab. 6.1: Distribution of samples with detection times above the threshold value

	2-3 hrs.	3-4 hrs.	4-5 hrs.	5-6 hrs.	6-7 hrs.
Number of samples detected within the given time span	1	5	15	3	1
% (n = 25)	4	20	60	12	4

Tab. 6.2: Distribution of samples with detection times below the threshold value

	4-5 hrs.	5-6 hrs.	6-7 hrs.	7-8 hrs.	8-9 hrs.	9-10 hrs.	10-11 hrs.	11-12 hrs.	12-13 hrs.	>13 hrs.
Number of samples detected within the given time span	1	3	5	11	12	10	4	2	1	26
% (n = 75)	1	4	7	15	16	13	5	3	1	35

Time limit 2: 7 hours. (All samples above the threshold value are detected within this period.)

Time limit 1: 5 hours. (The majority of samples above the threshold value are detected within this period.)

The total number of samples in each evaluation range (above/below the threshold value) is determined (*n*) and the number of samples in the time interval is calculated as a relative value (percentage) (tab. 6.1/6.2).

The time for the **warning value** (= the time within which all samples above the threshold lie = **yellow**) is easy to read off (seven hours in example above).

More flexibility is allowed when setting the cut off time for those samples clearly **above the threshold value (= red, cut off time)**. It is necessary to take into account both the majority of the samples detected within this range and the number of consequential “false positive” results.

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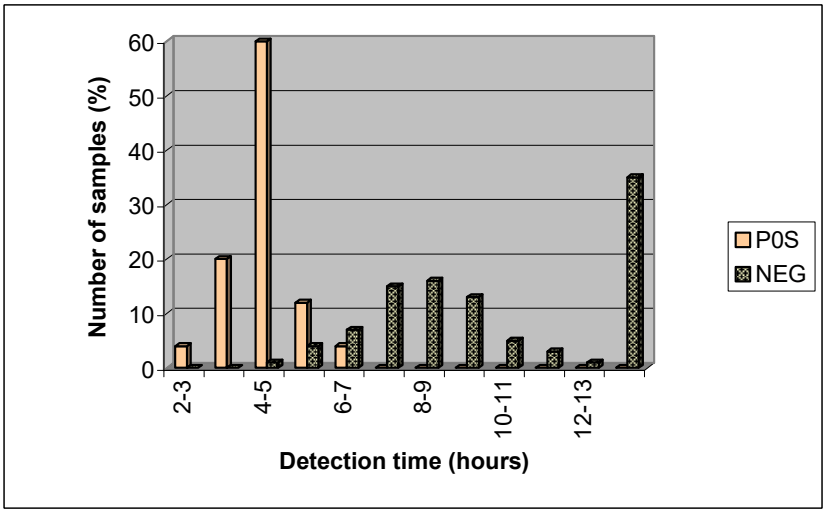
Fig. 6.3: Bar chart showing how time limits are obtained



Tab. 6.3: Distribution of results on the different time limits

84 % above the threshold value correct classification	**16 %** above the threshold value	**0 %** false negative
1 % false positive	**11 %** below the threshold value	**88 %** below the threshold value correct classification
***Time limit 1**:*	*5 hours* ***Time limit 2**:*	*7 hours*

At a value of seven hours for the warning value (= time limit 2) in the above example, 96 % of samples above the threshold value are correctly classified, 1 % of samples below the threshold value is incorrectly classified as being positive.

88 % of samples with results below the threshold value are correctly classified as lying below the threshold value.

The proportion of false negative samples is 0 %.

16 % of samples with results above the threshold value and 11 % of samples with results below the threshold value fall within detection times between five and seven hours.

While these results are very close to the threshold value, they indicate the need for a cut off time (time limit 1) of samples above the threshold value from those whose results comply in order to keep the proportion of samples with borderline results **(yellow)** within representative boundaries.

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7 Further investigations and confirmatory reactions

After a *BacTrac* measurement has been finished, the contents of the measuring cells (the enriched cultures) is available for additional investigations if needed.

Especially when investigating pathogens an additional confirmation of reactive samples is necessary.

7.1 Sub-culturing using conventional methods

Sub-culturing using conventional (standard) methods streaking out the loops of the enriched cultures from the measurement cells on different selective plates is the simplest way for clarification or time to time checks of impedance results.

In the case of investigating pathogens the selective enrichment is available in the measuring cell after impedance analysis has been completed and confirmatory reactions can be performed without any loss of time.

7.2 Biochemical reactions

7.2.1 The use of indicator substances

Some impedance media contain indicator substances for the additional evaluation of impedance results.

BiMedia 160C (selective media for coliform investigation) contains a pH-indicator for the detection of acid production from lactose by a colour change from violet to orange/yellow.

As an extra criterion for the evaluation of measurement results, the addition of chromogenic and/or fluorogenic substrates (i.e. MUG) to the growth media is possible in many cases.

7.2.2 Indoletest

Using *BiMedia* 160C the classical indole reaction (indole production from tryptophan) can be performed after addition of 1-2 ml of Kovac indole reagent directly to the measurement cell for reactive samples. In the case of presence of *Escherichia coli* a purple ring appears on the surface of the medium after only a few minutes.

The indole reaction can be used as an absence criterion for *E. coli*.

7.3 Immunological and molecular biological reactions

Especially for the detection of pathogenic microorganisms the combination of impedance technology as an initial screening and immunological resp. molecular biological methods as confirmatory assay of reactive samples has been proved valuable.

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The required enrichment of the pathogens up to 10^6 CFU/ml for immunological assays and for nucleic acid hybridisations is easily achieved by impedance analysis. False negative results can therefore be excluded very reliably.

Most of those methods can be performed directly from the enrichment culture present in the measuring cell. Salmonella enrichments with positive impedance signals can therefore be confirmed within minutes, e.g. by using immunochromatographic rapid tests.

While most immunological reactions are characterized by their rapidity, molecular biological confirmatory reactions have an unsurpassed specificity.

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8 Nutrient media for impedance microbiology

Microbiological nutrient media are particularly significant for use in impedance analysis. So for quantitative analyses in **universal media** it is not only important that the most diverse microorganisms from different product batches grow well, they must also yield comparable detection times in an impedance analysis. This comparability is only guaranteed, however, if all the separate components used, particularly the peptones and biological extracts, are absolutely compatible.

The same applies for **selective media**. A significant factor is the interaction between the growth-critical nutritive components and the inhibitors used. As selective media need to be fundamentally more selective for the impedance process in comparison with conventional selective media in order to achieve a corresponding specificity, the nutritive components must be selected especially carefully and matched together batch for batch.

The quality control of impedance media requires a multitude of investigations that go far beyond the usual measure of nutrient media analysis.

At SY-LAB every batch is investigated, with precisely defined test strains for characteristical impedance parameters such as *impedance detection time (IDT)* and *formation of growth signal*. Only after most thorough comparison of these parameters with the corresponding specifications and the results of a similarly investigated batch it is released for sale or rejected.

The *BiMedia* product line from SY-LAB continuously undergoes outstanding quality control which actually makes it possible to guarantee the impedance specific performance. These media which have been developed specifically for use in impedance analysis should be used for routine investigations to ensure a corresponding performance.

If using standard formulations or media developed in-house, these should be validated with diligence against a reference method.

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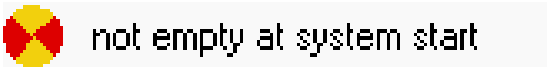
9 Troubleshooting

9.1 BacMonitor



PROBLEM:
Samples were inserted into the incubator before the initialisation cycle was finished and the status indicator of the incubator had not turned to green colour yet.

SOLUTION:
Remove the sample and wait for the next measurement cycle. When the status indicator turns green, re-insert the measurement cell again.

PROBLEM:
A contact error occurred during the measurement either permanently or just during a single or a few measurement cycles.

SOLUTION:
View the curve diagram for the corresponding position. If there is a non-typical curve with no or only a few measurement points missing, continue with the measurement. Sometimes the message will disappear right after removing the measuring cell and turning it by 180 ° and re-inserting it again.
If there is no curve at all there is a permanent contact error and the measuring cell as well as the electrode housings of the measuring positions have to be checked. Please contact an authorized maintenance engineer.

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PROBLEM:
A sudden tremendous increase in the measurement signal is interpreted as “curve too steep” because this seems impossible from a biological point of view.

SOLUTION:
View the curve and look when and where it happened. Using “DropStop” or drift compensation, the signal can be corrected if there was an influence on detection time.

PROBLEM:
The data received from the *BacTrac* incubator were defective either for just one or more measurement points.

SOLUTION:
View the curve diagram for the corresponding position. If there is a typical curve with no or only a few measurement points missing, the result was not significantly influenced by the measurement.
Check the connection of the serial connecting cable between the incubator and the PC (interface RS-232).
If the error message will persist for the following measurements an instrument failure must be considered. Please call an authorized maintenance engineer.

PROBLEM:
No measurement data have been received from the instrument.

SOLUTION:
Either the respective incubator position or the whole *BacTrac* device has an electrical error. Please call a maintenance engineer.

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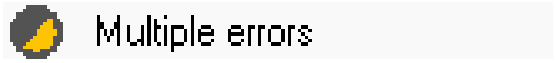
PROBLEM:
An error other than those specified above occurred.

SOLUTION:
Please call a maintenance engineer if this error message appears frequently.

PROBLEM:
The resistance value at the beginning of the measurement (M0) deviates strongly from the expected index value for the medium in use at the temperature defined.

SOLUTION:
The measuring cells are possibly calcified and should be put into a citric or acidic acid bath (5 %) for at least one hour.



PROBLEM:
Two or more of the errors described above have occurred simultaneously.

SOLUTION:
Please call a maintenance engineer if this error message appears frequently.

“Invalid variant type conversion.”

PROBLEM:
The selection area for “Analysis” has been activated without any confirmation.

SOLUTION:
Select an analysis and confirm by activating the confirmation button or not selecting an analysis.

A different application language appears on startup.

PROBLEM:
The language selection has not been saved.

SOLUTION:
Select the desired language again and tick the checkbox “Default language” within the “Open” dialogue to save this setting (see section 3.1.8.1)

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"Unable to connect to the database..."	PROBLEM: The directory "Database" has been renamed or removed from the application directory *"BacWin2"*. **SOLUTION:** **Check the name and/or the location of the directory "Database".**
"The table MEDIA could not be opened in the current database."	PROBLEM: The application does not start because the database specified does not contain a media table. **SOLUTION:** **Update the database specified with the most current update script (see section 3.1.4).**
"Error 7 : Hardlock not found."	PROBLEM: The *BacWin* software hardlock (dongle) is not connected to a port of the PC. **SOLUTION:** **Connect the hardlock dongle to the parallel port.**
"Hardlock drivers not found."	PROBLEM: The *BacWin* software has been installed without administrator rights, or the hardlock drivers supplied are not compatible to the operating system of your PC. **SOLUTION:** **Un- and reinstall the software from the supplied CD-ROM. If the problem persists please get the latest *BacWin* software which is compatible with the operating system of your PC and update your installation.**

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The function "Determine device" within the "Device configuration" window does not provide any serial number or the error message "buffer not empty" is appearing.	PROBLEM: The COM port buffers have not been emptied properly by Windows. **SOLUTION:** **Close the installation menu and start the instruments. The COM port buffers will be emptied. (The status indicator will remain red for a prolonged period of time.)**
The function "Device settings" in the "Settings" menu is not accessible (greyed out).	PROBLEM: The respective device has not been focused yet. **SOLUTION:** **Focus the device first to determine which one's settings are actually affected (see section 3.1.8.3).**

9.2 Device temperature

The current incubator temperature is significantly higher than the nominal temperature.	PROBLEM: The minimal temperature difference of 6 °C between ambient and nominal temperature is not met. **SOLUTION:** **Use air condition to decrease ambient temperature or connect a cooling device to the incubator.**
The current incubator temperature is significantly lower than the nominal temperature.	PROBLEM: The heating system of the incubator is failing or the temperature of the used cooling liquid is too low. (Only if a cooling device is connected.) **SOLUTION:** **Increase the temperature of the cooling liquid or reduce its rate of flow. (The temperature of the cooling liquid should be 2-5 °C lower than the desired incubator temperature.)** **If the heating system is failing please call an authorized maintenance engineer.**

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9.3 "BacEval plus"

No files found after selection.	Check the selection parameters and the date chosen.
Thresholds and grids are missing in B/W printed results.	PROBLEM: The drivers of your printer do not support the conversion of colours to B/W correctly. This problem is especially known to occur with HP laser printers. **SOLUTION:** **Use a colour printer or contact the supplier of the B/W printer**
"Unable to connect to the database..."	PROBLEM: The directory "Database" has been renamed or removed from the application directory *"BacWin2"*. **SOLUTION:** **Check the name and/or the location of the directory "Database".**
A different application language appears on startup.	PROBLEM: The language selection has not been saved. **SOLUTION:** **Select the desired language again and tick the checkbox "Default language" within the "Open" dialogue to save this setting (see section 3.1.8.1).**

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