Operation Manual

BioTrac 4250

Part 1: Device Manual

Part 2: Microbiological Manual

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Part 1: Device Manual

1 General

1.1 General warnings

The **BioTrac 4250** 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

1.2.1 Microbiological Analyser

The microbiological analyser BioTrac 4250 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. The BioTrac 4250 is a compact system, which, with capacity for 21 samples and an economical price tag, meets all those analytical needs, which could previously only be met with very costly equipment. Prefilled measuring cells and a broad medium range avoids the need for a complicated, extensively-equipped micro-biological laboratory and still delivers automatic and well-documented results along with numerous savings.

BioTrac 4250 combines a high sensitivity for detection of metabolic product changes with the possibility to use a big range of highly selective growth media.

1.2.2 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 the 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 the increase in the quality control have to be considered as well. The application of an impedance analyser such as the **BioTrac 4250** are nearly endless.

1.3 Warranty

SY-LAB Geraete 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 BioTrac 4250. 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.

2 Soft- and Hardware-Specifications

2.1 Technical data

Compact microbiological analyser for disposable measuring cells based on the impedance principle, suitable for direct and indirect impedance methods.

2.1.1 Housing

Plastic cladded metal construction with plastic roller blind for the protection of the keyboard and incubator openings. Docking station for Laptop PC.

2.1.2 Metal thermostat block

Incubators made of aluminium with holes to hold measuring cells, electrical contact from below, total capacity of 21 measuring positions, temperature range adjustable between 0 and 56° C, simple to clean due to self-contained holes, water cooling pipes.

2.1.3 Measuring cells

Direct impedance measurements for bacteria can be performed using 20 ml disposable plastic cells (polystyrene). 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.

2.1.4 Computer

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 USB for BacTrac 4250, 1 x USB for local printer (not necessary if network printer is available), screen resolution 1024 x 768 pixels, color printer, operating system Microsoft® Windows ™ 7, 8 (. 1) or 10 (Pro / Professional).

2.1.5 Software

Microsoft® 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.

2.1.6 Size and Weight

Dimension (W x H x D):	400 x 440 x 535 mm
Weight:	20 kg
Safety class:	The device confirms to safety class I

2.2 Normal Operating Conditions

Nominal power:	2 A {230VAC} or 4 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 according 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)

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 done.

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

2.4 Additional Specification

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 are about 10 years under normal environmental conditions.

2.6 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

2.8 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.

2.9 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.

2.10 General Safety Precautions

During operation, maintenance or any other repair work, the following special safety instructions have to be followed.
For your own safety and for the operational safety of the device, please read these carefully before using your device.
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 BioTrac 4250 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.
- Always follow all warnings and instructions that are affixed or noted on the device itself.
- 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 block of the incubator 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. 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.
- 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.

2.11 Parts description

Fig. 1 Top view

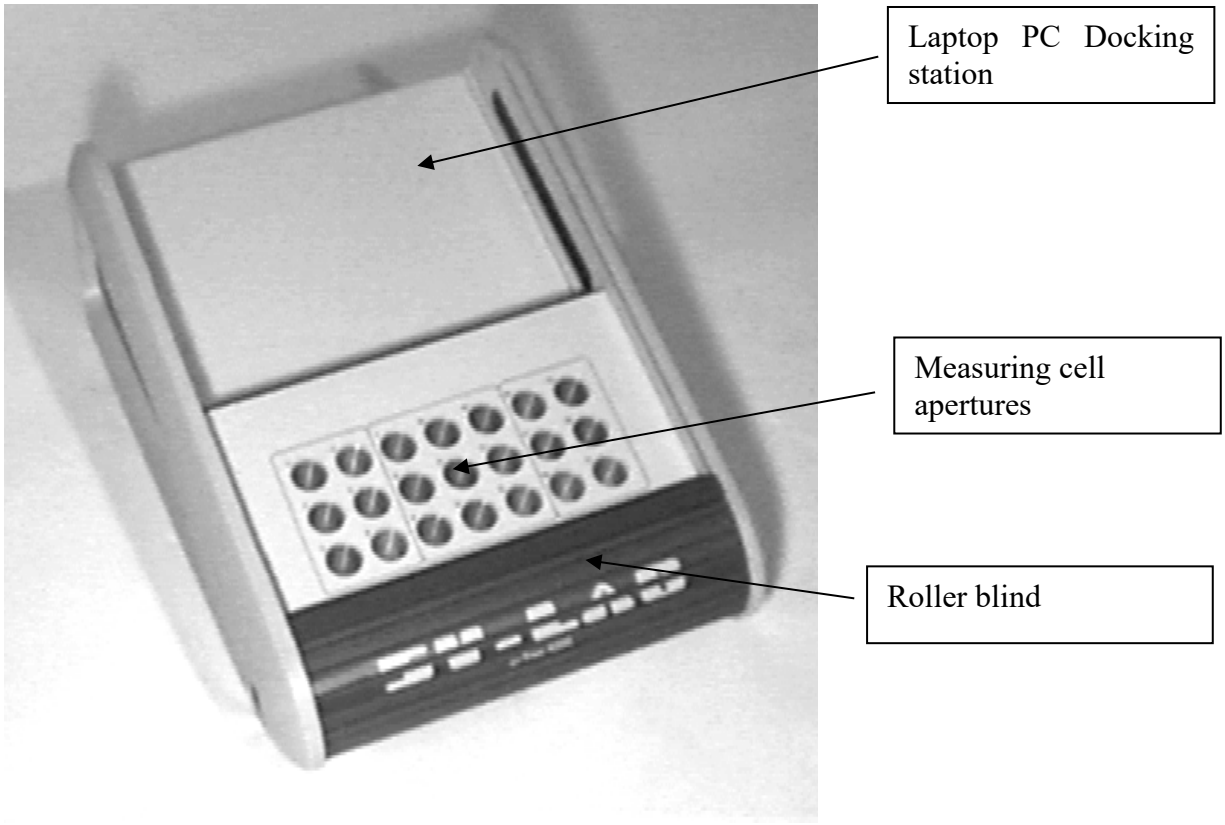
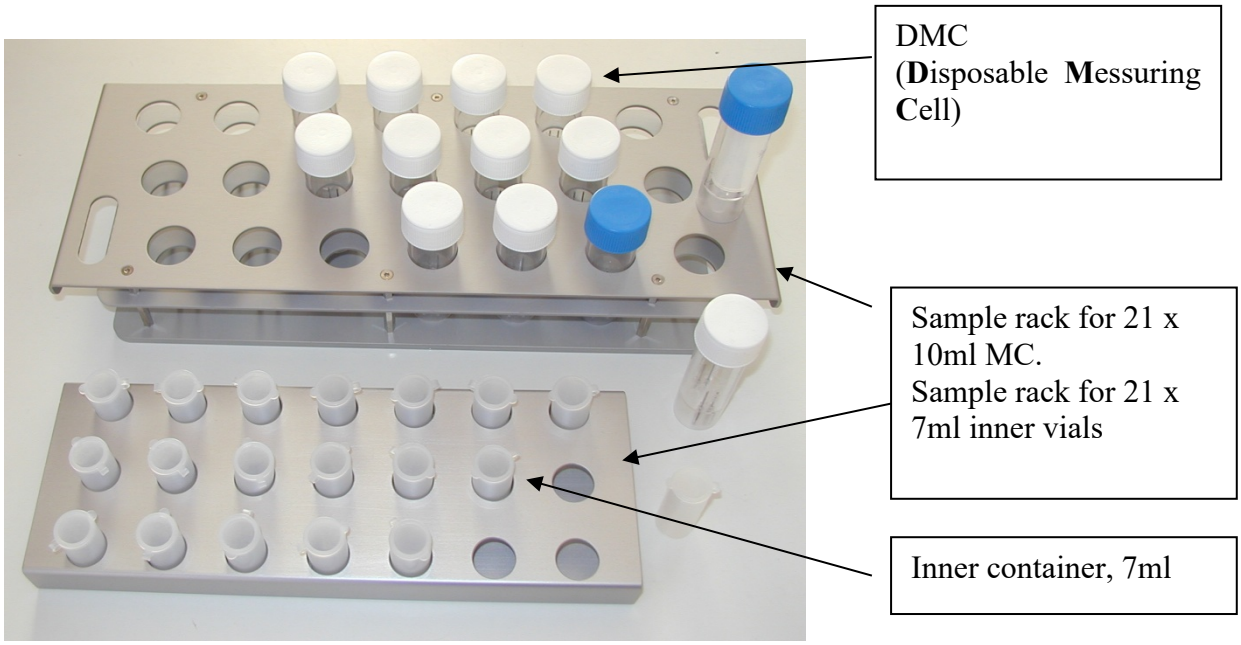


Fig. 2 Sample rack



2.12 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.

2.13 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% 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.

3 Installation

3.1 Unpacking

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

When unpacking the device, check that all the parts shown are present and undamaged.

Pos.	Off	Art.no.	Description
1	1	40-425010	Device without PC
2	1	40-421700	Measuring cell rack 21, for10ml MC
3	1	40-421730	Cooler connections kit
4	1		Laptop support plate
5	1	40-421760	Temp. measuring cell, BioTrac
6	1	40-421740	Cleaning case
7	1		Power connector
8	1		USB cable
9	1		Software CD ROM
10	1		Operating instructions manual

Note: please inform us immediately if any parts are missing or damaged.

Lift the device out of the packaging using the marked lifting points. The device must never be lifted by the roller cover.

Do not destroy packaging after unpacking.
It is a part of your device. Please retain for safe transport at a later date, should this become necessary.

***Take care*:** check the voltage values given on the device label with your mains power supply. You must not activate the device unless the two values are the same. If they do not agree, contact your dealer or SY-LAB Austria immediately. The supply voltage is factory set and cannot be altered by the user.

3.2 Installation of laptop support plate

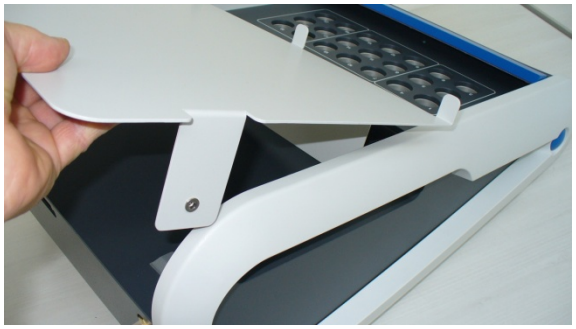
For laptops exceeding a width of 330 mm (~13 in), an additional Laptop Support Plate is required.

- Unpack and check contents
  1 pc. Support Plate
  2 pcs. Shims (black washers)
  2 pcs. Screws

Fig. 3 Content of laptop support plate package

- Position the Support Plate over the analyzer as shown below.

Fig. 4 Positioning of support plate



- Next, mount the Support Plate with the supplied screws and shims onto the analyzer as shown in the pictures below.

Fig. 5 Assembly of support plate

Fig. 6 Device complete with support plate

3.3 Device connections

Electrical connection - BioTrac

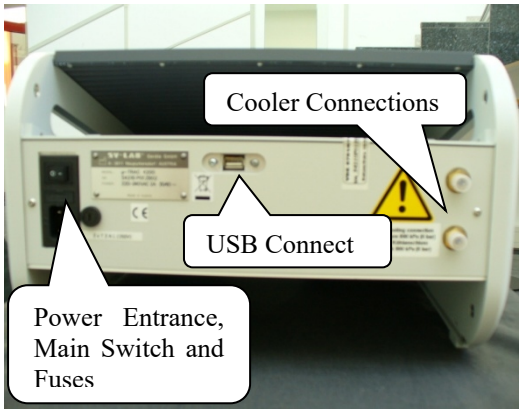


Fig. 7 Rear view of device

The power input is located on the back of the instrument. Use only the power cable supplied with the instrument.
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.
Next to the power inlet, you will find the main switch as well as the fuse compartment. When operated at 230 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 instrument type plate)

Electrical connection – computer / laptop

The BioTrac will be operated by software and requires a proper computer to function correctly (see specification).

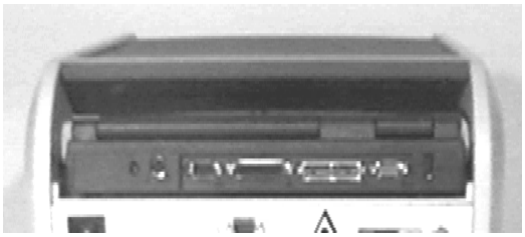


Fig. 8 Laptop

USB connection: BioTrac to PC

The **BioTrac** is connected to a **PC** via the USB interface.

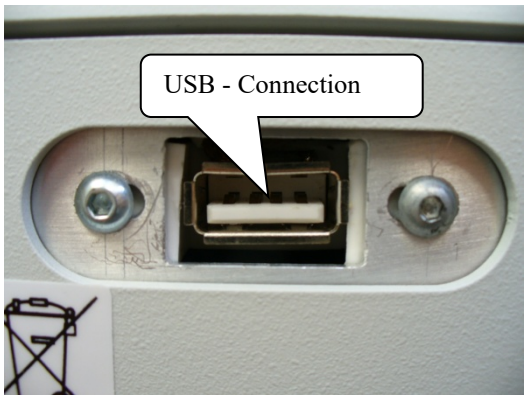


Fig. 9 Interface

The **BioTrac** will be detected automatically.
Only the PC allocated to the **BioTrac** may be connected. Use only the communication lead provided with the instrument.

Cooling system connection

Two cooling system connectors can be found on the back of the **BioTrac**

Fig. 10 Cooling system connectors

These are used as required for counter cooling (if the incubator temperature is nearly the ambient temperature (approx. 7°C) or for cooling the incubator block. The incubator block can be used within a temperature range of 0–56°C.
The direction of flow is not significant.
Only minimal counter-cooling is generally necessary. If the coolant temperature is too low or the flow rate of the coolant is too high, the heater may be overloaded. As a result the system may not reach the pre-set temperature.

Cooler Connection Kit

The **BioTrac** device is supplied with a cooler connection kit comprising the following parts:

1x ball tap with short hose connector and elbow
3m cooling connection hose
2x hose clamps
1x M10x1 elbow
1x open-ended spanner, 7 & 8 mm
1x open-ended spanner, 12 & 14 mm.
(see Fig. 11)

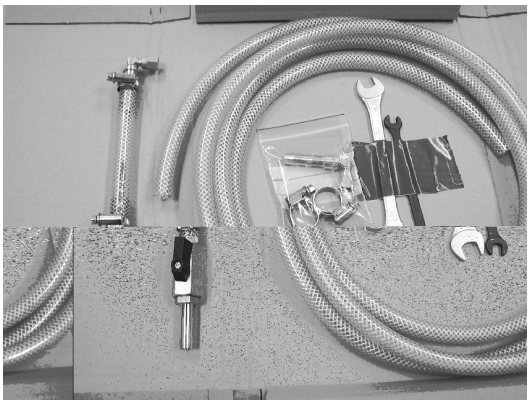


Fig. 11 Cooler connection kit

If the cooling system is to be used, connect as shown in the figure below.

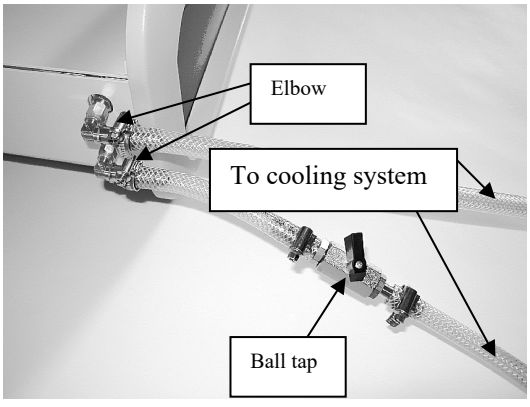


Fig. 12 Hose connection

There is no need to observe a particular direction of flow.

Fuses

The main fuses are located at the mains input on the device (230V devices are protected by a 5x 20 mm x 3.15A slow-blow, 250V fuse). One of the fuse holders is located between the main switch and the mains input. The other right next to it.

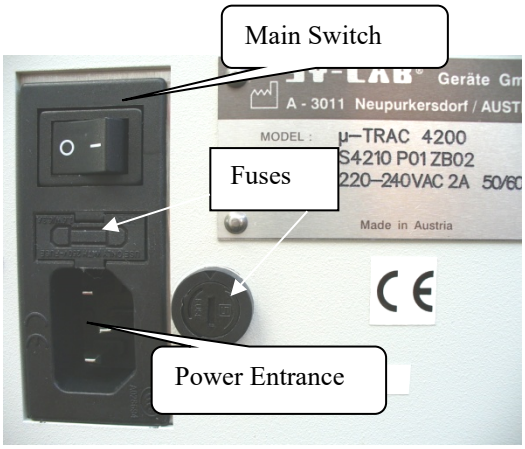


Fig. 13 Mains input

4 Cleaning

Description of cleaning set

Fig. 14 Cleaning Set

Fig. 14 shows a complete set of cleaning tools. Use only the tools that are recommended by SY-LAB. Other tools might damage your instrument.

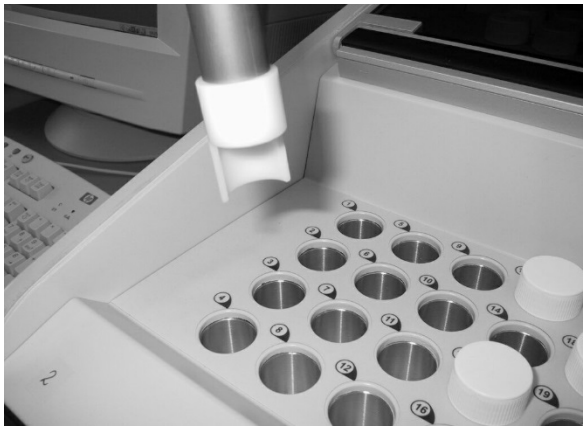


Fig. 15 Contact Socket Cleaner

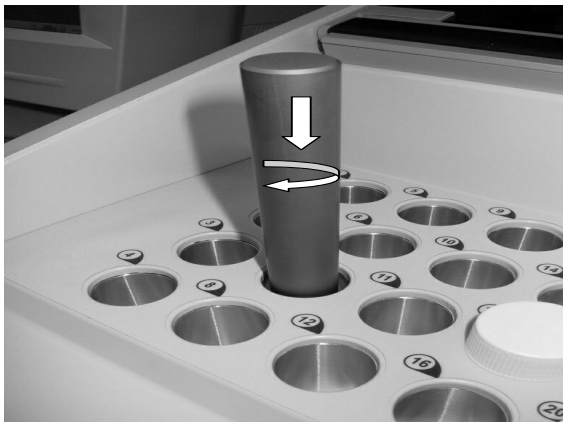


Fig. 16 Extender Tool

The tool in **Fig. 15** and **Fig. 16** 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.

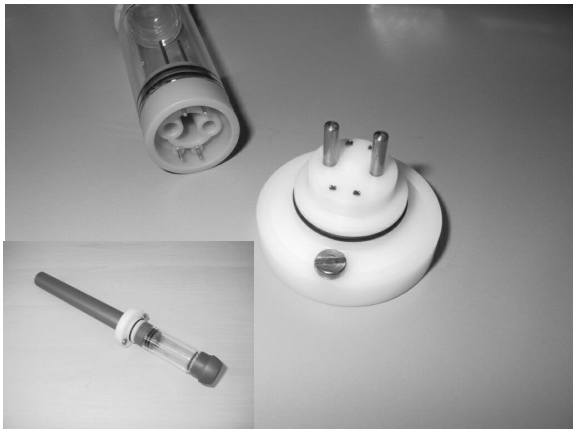


Fig. 17 Contact Socket

Fig. 17 shows a contact socket. It is used as a holder for assembling or disassembling measurement cells. The insert shows the tool with the measuring cell. When a measuring cell is dismantled, always replace the O-rings.

Fig. 18 Pliers

The pliers in

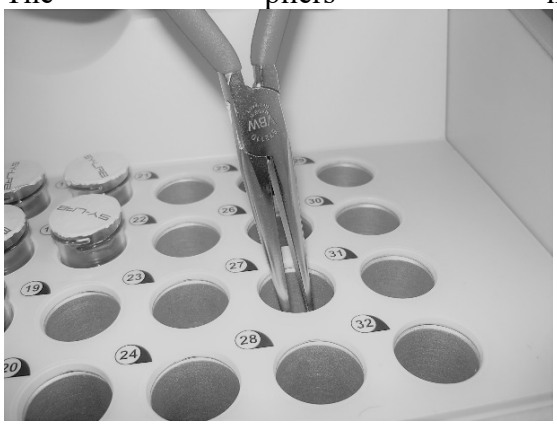


Fig. 18 are useful to pull out any contact sockets from within the incubator position in case the measuring cell came apart.

Fig. 19 Guiding Rod Scrapper

The tool in **Fig**. **19** is used for removing stains and crusts of dirt from within the contact socket.

Fig. 20 Milling Head f. Meas. Position

The milling tool in **Fig. 20** is used for scrapping off any dirt on the walls and bottom of the measuring position.

Fig. 21 Scrapper for Contacts (1)

Fig. 21 and **Fig. 22** shows how the contacts of a contact socket is cleaned using the contact scrapper.

Fig. 22 Scrapper 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.

5 Appendix

5.1 Accessories

40-421700	Sample preparation rack to hold 21 x 10ml measuring cells
40-414735	Sample preparation rack to hold 21 x 7 ml measuring cells (inner vials)
40-421740	Cooling connection kit – necessary if set incubation temperature differs more than 5°C from ambient temperature)
40-419899	Cleaning set
40-421760	Temperature gauge, NTC 1000 Ohm for 10ml incubator

5.2 Consumables (details see media list)

- Disposable measuring cells, sterile
- Disposable measuring cells, sterile, filled with nutrient (see media list)
- Yeast/mould -indirect measuring cells (limited re-usability)
- Inner containers for yeast/mould measuring cells

Part 2: Microbiological Manual

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.

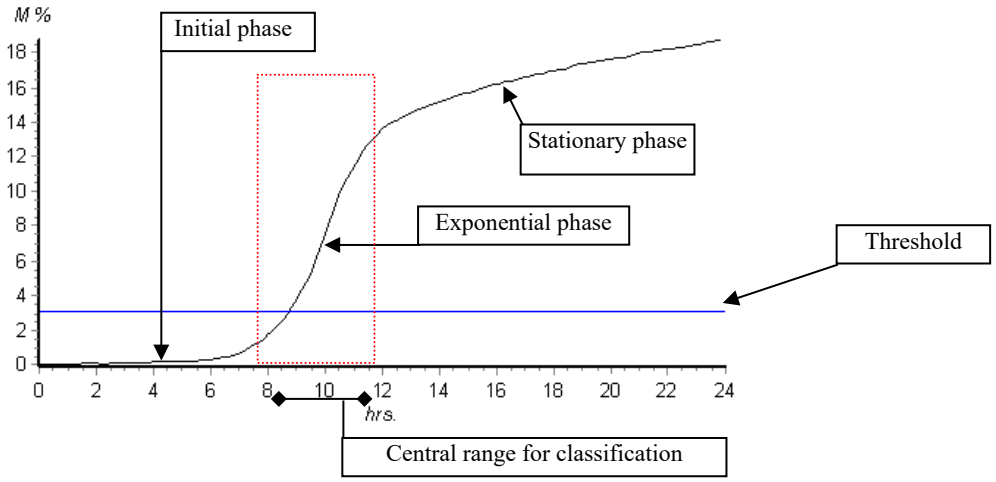
This decrease in electrical resistance can be measured using at least two electrodes introduced into the nutrient solution. If an AC voltage is applied to the electrodes, the decrease in the so-called **impedance** in the AC field can be measured.
The *BioTrac* measuring technology (see section 2.1) 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 *BioTrac* measurement curve can be divided into 3 characteristic ranges:

- Initial or adaptation phase
- Exponential phase
- Stationary phase

Fig. 1.1: Typical impedance signal



During the **initial** or **adaptation phase** the microorgasms 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**.

The point of inflection of the curve and the earlier exponential phase, respectively, have 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 their 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, the shorter the length of time before the formation of the characteristic measuring curve.

The few microorganisms of only lightly contaminated sample materials - and the *BioTrac* 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 evaluation 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 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 a signal change 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

2 *BioTrac* 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 *Siemens; S*) and to **admittance** (= reciprocal value of the impedance, measurement unit *Ohm, Ω*).

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:

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

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 little or uncharged, 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 3 molecules of oxygen (O_2) to 3 molecules of CO_2. 3 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).

Greatly simplified, the *BioTrac* 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 BioTrac signal development

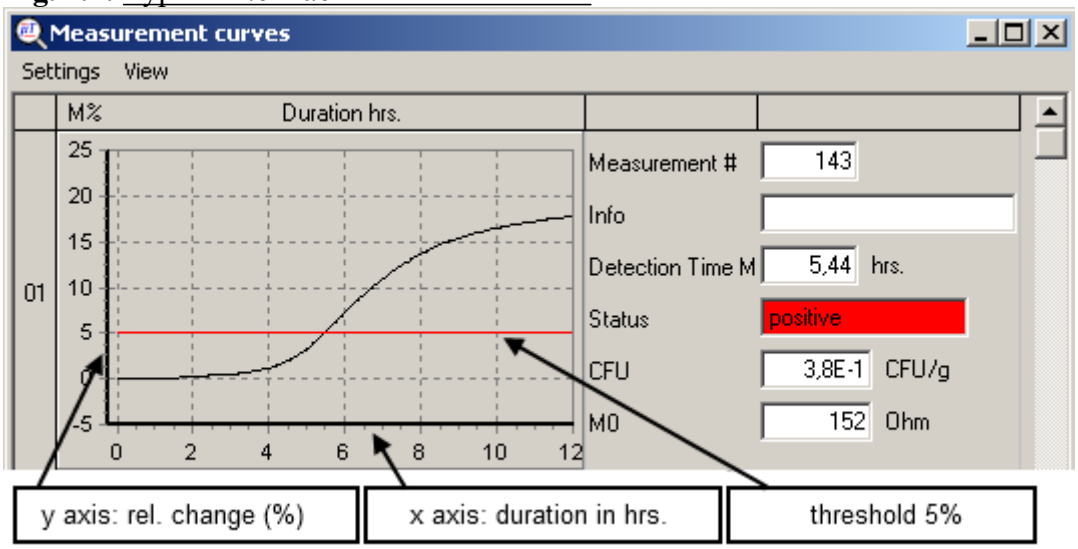
The *BioTrac 4250* measuring device provides relative values. The change in the M-value is therefore the relative change in the reduction of the media impedance (in percent) in relation to the starting value.
The relative measurement offers the advantage that all measurement curves have an identical

starting point, thereby eliminating product specific fluctuations in the starting conductivity.

The device shows the **relative change in the measurement signal**, displayed in **%M** (M= media impedance) for the duration of the set measurement period, whereby the measured variables are recorded at a minimum of 2.5 minute intervals (this value can be altered).
Because of the high-resolution measurement electronics changes of 0.5 - 1% can be considered as being significant

.
Figure 2.1 shows a typical measurement signal.

Fig. 2.1: Typical *BioTrac* 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.

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.

3 Commisioning the device and setting the measurement parameters

3.1 Commissioning the device

3.1.1 Installation of the MicroTrac measurement and control software

The MicroTrac 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 automatically by inserting the CD into the drive of your PC. The software will be installed in a directory called "SY-LAB\MicroTrac2" of your local Windows system drive. Besides the main applications MicroTrac and MicroAssist, device 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 MicroTrac 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\MicroTrac2", assuming drive C: to be the Windows system volume.

If the PC system has been provided by SY-LAB Geraete GmbH, the MicroTrac 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.
- USB ports: One for *BioTrac* device.

The use of an uninterruptable power supply (UPS) for *BioTrac* and PC is strongly recommended! The *BioTrac* unit including the PC can be sustained by a 2200 VA unit for approx. 60 minutes.

3.1.2 Installation of the *BioTrac* incubator (Hardware)

Connect the *BioTrac* incubators with the delivered main cord to your power line.
Using the provided USB cable connect the *BioTrac* incubator to any free USB port of the PC.

☞ 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.

☞ During the installation process of the MicroTrac software the corresponding USB drivers for *BioTrac* are installed as well. From this moment forth the instrument will be detected correctly by the operating system.

After connection to the PC and power line, the *BioTrac* incubator is ready for use and can be switched on using the on/off switch on the back of the device.

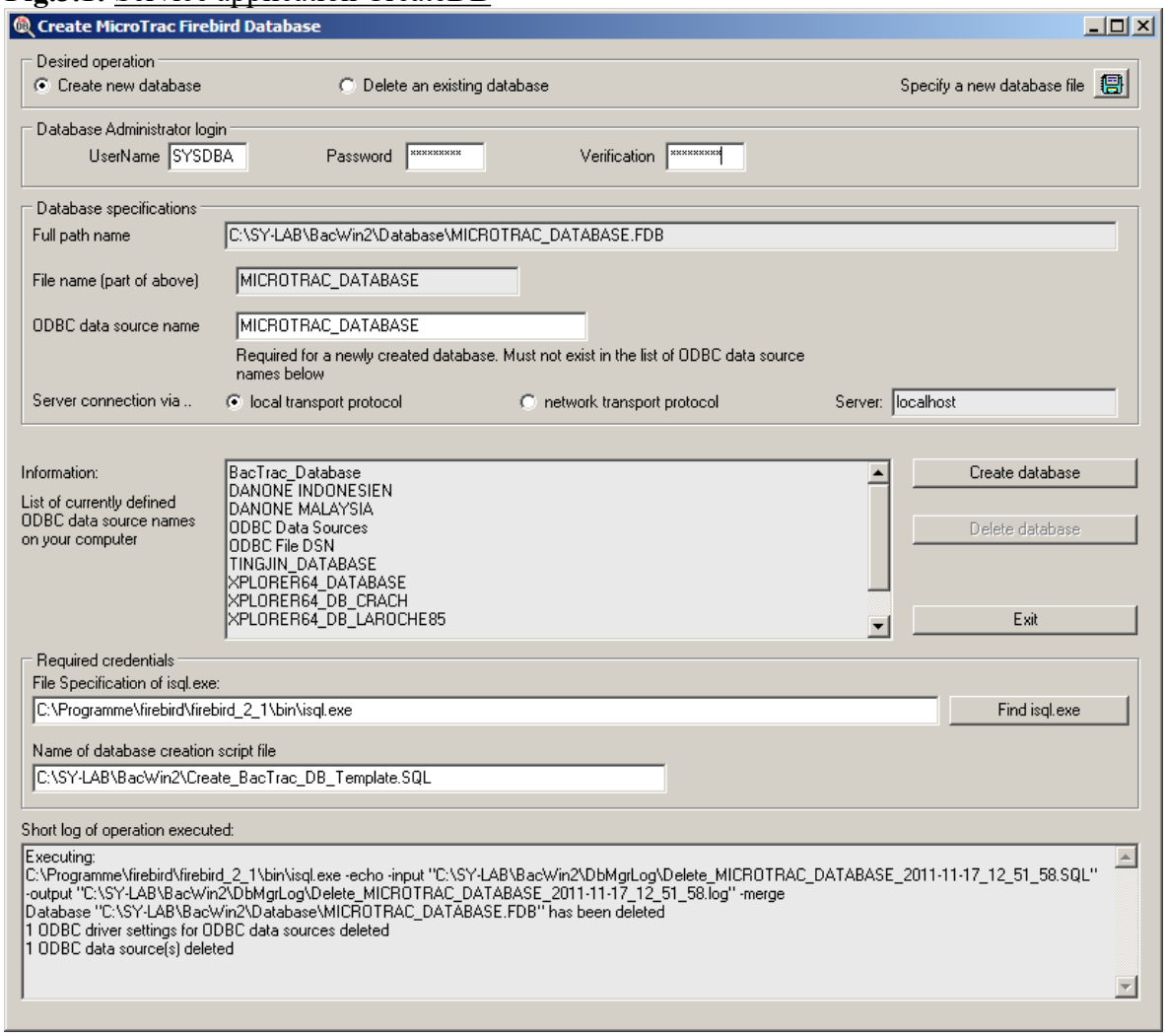
3.1.3 Creation of a database

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 “MicroTrac2”.
2. Select button "Create new database“.
3. Specify database file name with „Specify a new database file“. (A standard “Save” dialogue is shown.)
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!”**. (MicroTrac 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 the drive 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 located on a local drive. The usage of a network drive is not recommended.

Databases are not deleted by deleting the associated files! Instead also use the application CreateDB for this purpose. Deleting works in analogous manner to setting up the database by choosing „Delete an existing database". The file name needs to be entered in an "open" dialogue along with username and password of the database administrator.

3.1.4 Update of an existing database

When the MicroTrac 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 "MicroTrac2".
2. Specify update script (currently "Update_MicroTrac_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!"**. (MicroTrac 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 periodical 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\MicroTrac2\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!"**. (MicroTrac 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 "save" the settings are stored in an .ini file and become valid.

☞ To automatically start DBBackupService when the system is started a link to the application has to be put into the program group "Startup".

☞ The application creates backups according to the defined time plan regardless of whether MicroTrac is running (or has run in the meantime).

☞ A new file is created in the target director for each backup time. If these files are not deleted after a certain period of time, eventually an overflow can 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 "MicroTrac2" and select the option "Backup".

Additionally the following information has to be provided:

- Database to be backed up (usually located in C:\SY-LAB\MicroTrac2\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 (yet), enter the username of the database administrator **"SYSDBA"** and the password **"SyFbBMX!"**. (MicroTrac 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 has been corrupted and cannot be accessed anymore by MicroTrac software it is required to restore the latest backup to the database.

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

☞ Before restoring a backup MicroTrac and MicroAssist_plus must be closed if they are currently running.

Start DBBackupCreator (via Start/Program files/SY-LAB/MicroTrac2) 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\MicroTrac2\Database\MICROTRAC_DATABASE.FDB).
- Destination of latest backup file.
- 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!"**. (MicroTrac 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" and confirming the warning message the backup is carried out.

3.1.8 MicroTrac Software – Preparation of instruments for the measurement

The MicroTrac software consists of two parts:

1. The MicroTrac Measurement Software **(MicroTrac)**
2. The MicroTrac Data Evaluation Software **(MicroAssist)**

The MicroTrac **measurement program** is the part of the software that is used for the detection of measured variables and data management and as such it represents the central part of the program.

After opening this program you can install and/ or activate individual incubators, adjust all measurement parameters and manage ongoing measurement cycles.

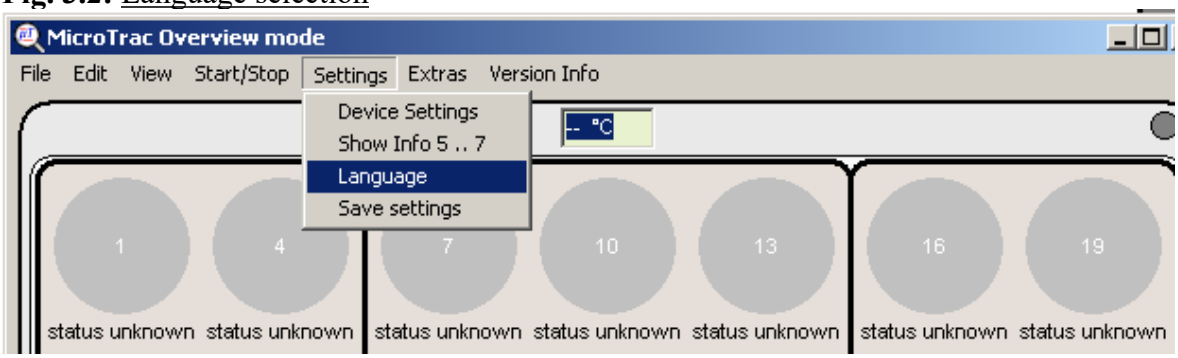
At the start of the program you will see the MicroTrac Overview window.
Before adjusting measuring and analysis parameters, selection of the language and alignment (installation) of the already connected incubator to the virtual serial port becomes necessary.

3.1.8.1 Selecting a language

The program language can be selected from the "MicroTrac Overview mode" menu in the "Settings" option under "Language". The corresponding *.LNG files can be found in the MicroTrac2 program directory.

After selection of a language the function "Save settings" must be selected for permanent storage of the language selection. The standard program language is English.

Fig. 3.2: Language selection



☞ If the language selection has not been saved using "Save settings" the standard program language (English) will be loaded on the next start up of the program.

3.1.8.2 COM port alignment (device configuration)

When the instrument drivers are installed during software installation a COM port will be distributed to the *BioTrac* device.
Alignment of COM port to the instrument will be performed in the "MicroTrac Overview mode" program window by selection of the function "Installation" within the "Extras" menu. After selection of "Installation" the Device configuration window will be opened and all available COM-ports will be displayed. Select the appropriate COM port there.

After selection of "Query device" the serial number and/or the internal software version of the connected instrument will be automatically displayed.

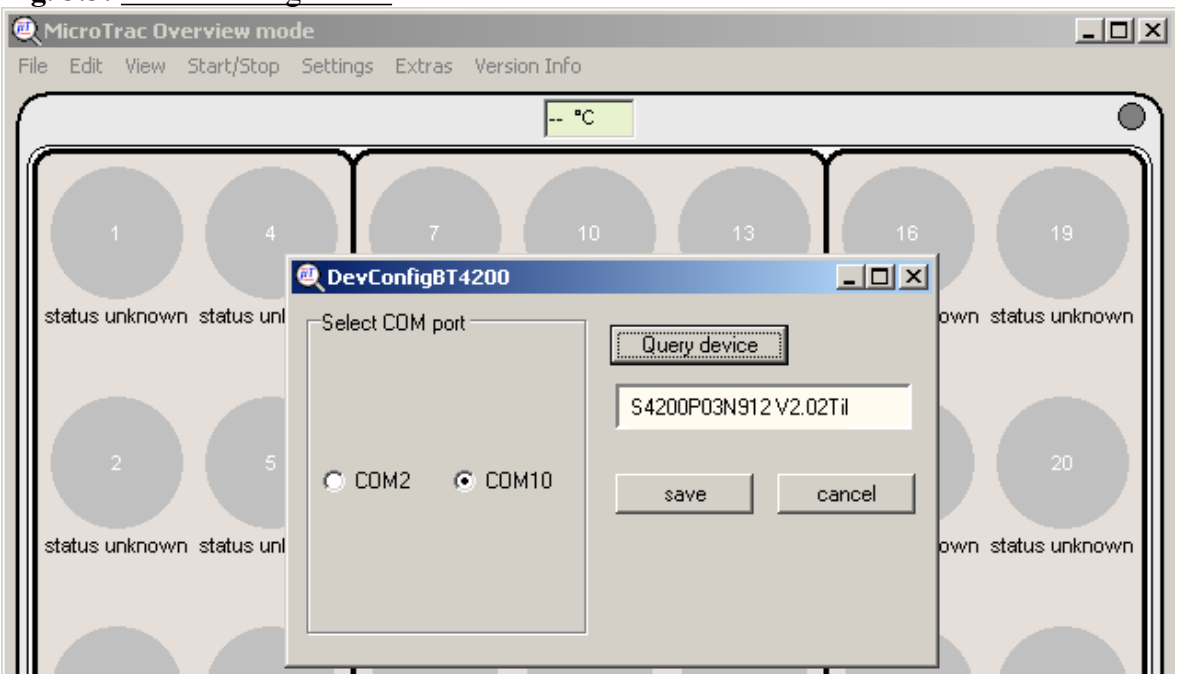
- ☞ For a proper determination the instrument must be connected to the PC in the right way and switched on.

When the instrument has been aligned with the correct COM port the configuration must be stored by selecting the "save" button.

Performing the device configuration is only necessary upon the first putting into operation of the system.

- ☞ The device configuration can only be changed when no measurements are ongoing (i.e. the program must be inactive).
- ☞ If the device configuration is changed, incubation temperature and parameters **for all positions** must be set anew. **Attention:** This is necessary even if the old settings are still displayed!

Fig. 3.3: Device configuration



3.2 Starting the instrument

The menu point “Start/Stop” within the “MicroTrac Overview” window is used to activate the connected incubator (“Start instrument”).
After the instrument is started, the device status display (circle in the right upper corner of the incubator displayed in the MicroTrac Overview window will change its colour to yellow (initialising).

After a few seconds the device status display will change to a green colour and the instrument is now ready for insertion of samples.

☞ After selection of “View/Legend” in the MicroTrac Overview window all the different symbols for the device status display, position display and error messages can be reviewed.

3.3 MicroTrac “Watchdog”

A “Watchdog” monitoring program is included in the software package.
Every time the *BioTrac* device is started the “Watchdog” is also started automatically and monitors the status of MicroTrac. In case that MicroTrac crashes or freezes during measurement the “Watchdog” automatically restarts the program restoring the status before the crash and all measurements continue.
As soon as the *BioTrac* instrument has been stopped the “Watchdog” is also shut down. Note that shutting down MicroTrac via the menu stops the instrument and thus irreversibly concludes all ongoing measurements.

In case that the PC is shut down or restarted unintentionally during measurement the “Watchdog” is shut down. In this case the measurements will resume as soon as MicroTrac is restarted by the user which will also reactivate the “Watchdog”.
To ensure an automatic restart with continuation of measurement after a power loss the following steps have be followed: a) MicroTrac is put into „Startup“, b) PC is configurated for automatic booting after power loss and c) automatic Windows login is activated.

In any case, the PC should be connected to a UPS unit since a smooth restart of MicroTrac after a power blackout cannot be guaranteed.

3.4 Setting of measurement parameters

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

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

- ☞ If for the first measurement these parameters 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 identification info 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 identification info was already cleared when the measuring cell was removed.

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

3.4.1 Setting of the *BioTrac* incubator temperature and measurement interval

The incubation temperature has a big impact on the generation time of microorganism with direct influence on enzyme activities and impedance signal generation and is therefore of high 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 MicroTrac Overview mode window will open a window for setting of incubator temperature and measurement interval for the focused incubator (Fig. 3.5a). In the lower part of the incubator temperature setting window the serial numbers and the internal (incubator) software version will be displayed.

Alternatively, this function is also accessible by a double click directly into the small window within the incubator where the incubator temperature is displayed.

The desired reference temperature is adjusted using the arrow keys by 0.1°C each time.
The temperature setting can also be selected and entered directly via the keypad.

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

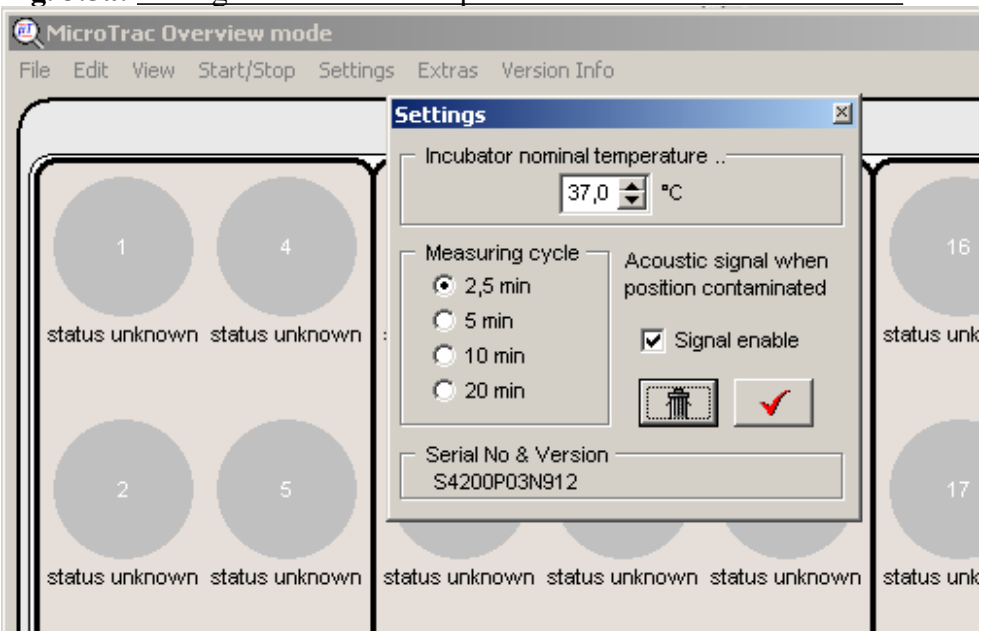
- ☞ If the temperature will be decreased, stabilisation may last longer if no counter-cooling is used by usage of the cooling adapters on the instrument back side.

The measuring cycle time (= interval time) defines the time span within every position will

be measured again. As standard value 2.5 min is recommended. Only for measurements lasting longer than 72 hours this value should be increased, to record less individual measurement points and save disc memory.

After entering the new parameters have to be confirmed by pressing the “OK” button. When selecting the “CANCEL” button, the last changes are discarded and the software reverts to the temperature used for the last measurement.

Fig. 3.5a: Setting the incubator temperature and measurement interval



☞ Temperature constancy can be achieved only if the difference between the set "REFERENCE” temperature and the ambient temperature in the room is at least >6°C. Otherwise, active counter cooling is required, via the cooling connector on the back of the device, using a circular cooler to achieve the required temperature setting.

3.4.1.1 Calibration of the BioTrac 4250 temperature sensors

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

Required materials:

- A calibrated thermometer
- 1 measuring cell filled e.g. with water
-

For Temperature calibration, the following process is recommended:

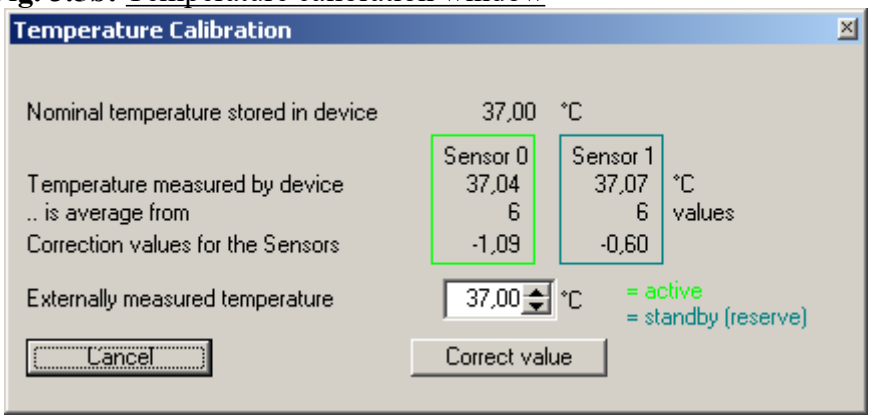
- Turn on the device
- Start the MicroTrac program
- Fill the measuring cells with water and put it into position 19 of the incubator.
- Wait at least three hours for the temperature to be distributed evenly across the block and also the cell to reach its final temperature.
- Put in the sensor of your calibrated thermometer into the control cell of the first

incubator, close the cover as far as possible and wait approx. 1-2 minutes before reading off the result.

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

After this preparations, please choose the menu **Extras/ Temperature Calibration**. The following window (Fig.3.5b) will appear:

Fig. 3.5b: Temperature calibration window



The *BioTrac* device has two temperature sensors, one of them is the active sensor (which is used for the temperature control), the other one is standby in case the first sensor is defective.

For the calibration it is necessary that an average of at least 6 consecutive temperature measurements can be calculated, otherwise the Correct Value button will be greyed.
Please set the "**Externally measured temperature"** field to the reading of your thermometer and then press the Correct Value button. In turn, the fields **".. is average from"** will be reset to zero, and Correction values for the sensors will be recalculated. As a consequence the "**Correct value"** button becomes grey (until 6 consecutive measurements are available again).

NOTES:
1. The value of the standby sensor will be sent only in the measuring pauses, so it might be necessary to wait until the current cycle has finished obtaining the required 6 readings for the standby sensor.
2. It is not required 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. The most frequently used measurement temperature should be selected for sensor re-calibration.

3.4.1.2 Using the temperature measuring cell

In the temperature measuring cell, the electrodes are jumped with 2 high temperature-resistant resistors.

The temperature measuring cell can be placed in any *BioTrac* measuring position.

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; ± 0.5°C, in the incubator, ± 1.5% M fluctuations in the measurement signal in 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 are approximate 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 "MicroTrac Measuring Parameters" menu (cf. Fig. 3.6)

To open the menu use the function key F6, or you can select "View/parameter setting" in the MicroTrac Overview window. Alternatively, you can open the MicroTrac measurement parameters menu by a double-click of the left mouse button outside of the circle resembling a measurement position.

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 duration times for active measurements that are less than the time span already gone since the end of the warm up time.

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

According to the pre-set duration, the ring diagram in the menu "MicroTrac Overview" 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 dependant upon 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 threshold value is exceeded more quickly / the impedance detection time is shorter, the higher the contamination level of the sample.

3.4.3.1 Selection and setting of 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 **MicroTrac Measurement parameters** menu option.

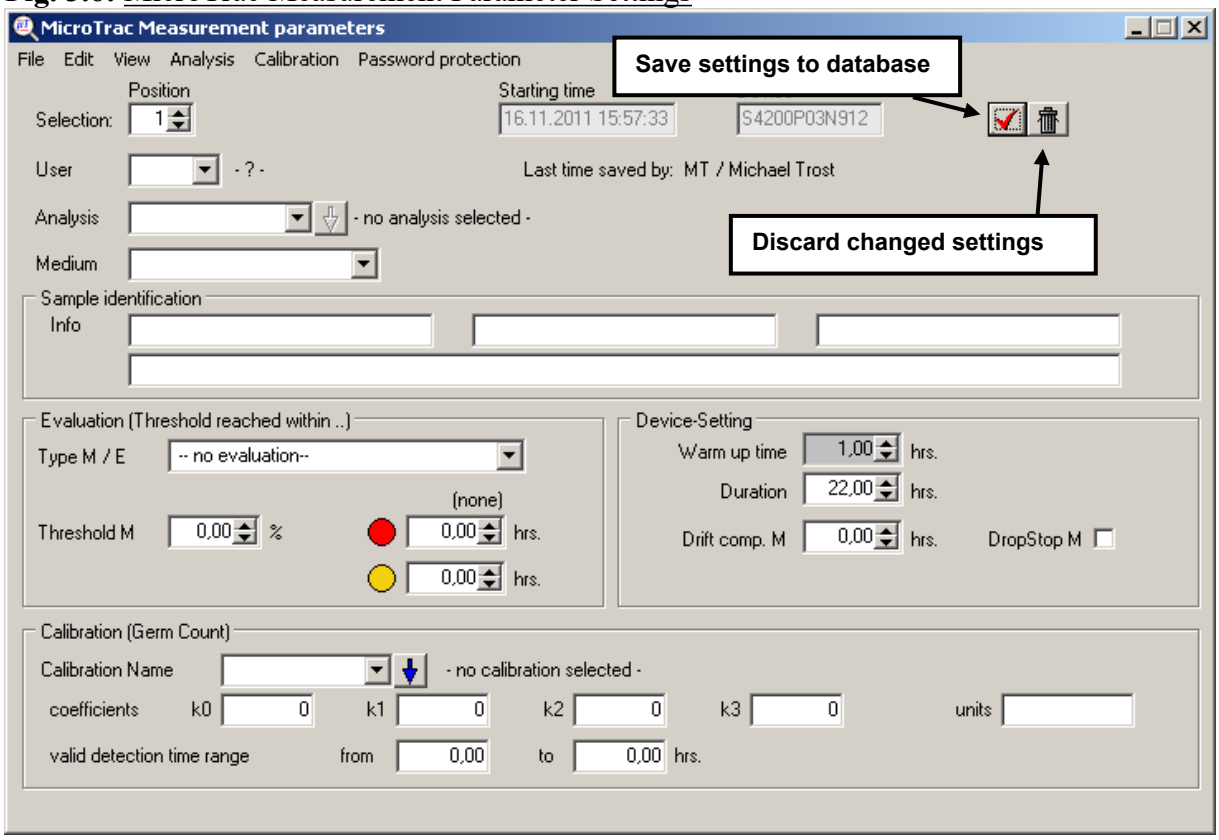
If there is no threshold value given for the required application, we recommend setting the threshold value to between 3-5% M.
The lowest possible threshold value can be determined empirically via the investigation of several sterile samples. It should be set high enough so as not to be interrupted by 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 need not be set separately when using the pre-set MicroTrac software analysis settings. These are accessed via the “MicroTrac Measurement parameters/ analysis” menu option.

- ☞ To compare measurement results it is absolutely essential to use a common threshold value

- ☞ .For advanced copy possibilities for simultaneous alignment of measurement parameters to different positions see section 3.4.9

Fig. 3.6: MicroTrac Measurement Parameter Settings



Position:
By the use of the scroll bar or by direct entering the Position No., the individual incubator position can be selected for entering the measurement parameters.

Analysis:
By the use of the scroll bar pre-defined analysis types can be selected. To fill in the parameters of an already selected analysis it is necessary to activate the blue 🡻 beside the selected analysis. If the analysis selected is not confirmed within 5 sec. the parameters will disappear.

Medium:
By the use of the scroll bar the medium used and the preset temperature can be selected. If the medium is selected the program compares the nominal M0 value with the measured value at the start of the measurement and in case of a larger deviation the error message “M0 exceeded” is shown.

Calibration:
By the use of the scroll bar a calibration (if available) can be selected. To fill in the parameters of an already selected calibration it is necessary to activate the blue 🡻 beside the selected calibration.
It is also possible to use calibration parameters (coefficients) directly entered in the respective fields.

Evaluation:
By the use of the scroll bar, the type of automated evaluation (red/yellow/green) according to pre-defined TVC limits or presence/absence parameters can be selected.

Sample identification:
For text fields for entering the sample description are available.

User:
A user must be selected to perform an analysis

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. (since 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 1 hour. During this time the set incubation temperature in the measuring cells is guaranteed to be reached.

Shorter warm-up times are possible if the media has been pre-adjusted prior to inoculation with the sample. Again, care must be taken to ensure that routine analyses are carried out under constant conditions.
The warm-up time is entered in the "Measurement parameters" menu for the device setting (see fig. below)

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

- ☞ If no other warm-up time has been set when the measuring cells are put in place, the default warm-up time of 1 hour will apply.

- ☞ The lowest possible limit for the warm up time is 1 hour.

- ☞ THE WARM UP TIME MUST BE SET BEFORE A MEASUREMENT CELL IS INSERTED INTO THE INCUBATOR (i.e. for the empty position) OTHERWISE THE DEFAULT WARM UP TIME OF ONE HOUR 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.

NOTE:
Any change in the measurement parameter window appears in red colour and needs to be confirmed by the function “Save settings to database” before it will be used.

3.4.5 Evaluation

Analysis results can be simultaneously classified by the MicroTrac software.

Automatic classification dramatically simplifies evaluation. It is possible to divide the measurement results into **2 categories (red/green**) or **3 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 3 classes) in the form of:

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

3.4.5.1 Selection and setting of evaluation criteria

The classification criteria are basically determined using time thresholds

If in the *Measurement parameters* menu option (see Fig. 3.6 below) a single time threshold has been entered 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 for yellow has been set differently from the one for red, three categories of classification apply:

Measuring positions marked **green** are those whose impedance detection time (IDT) is greater than the threshold time given for yellow.
Samples are evaluated as **yellow** when their IDT is greater than that for red, but lower than the threshold time given for yellow.

Finally, all samples whose detection time is lower than that set for red, are evaluated as **red**.

These colours can be allocated very easily 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

There are three different evaluation types that you can choose from:

1 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 threshold but will not be saved to database.

2 M: M-threshold + time

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

3 M2rel: **M-threshold + time, relative values**

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

3.4.6 Further Settings within the Measuring parameter menu

3.4.6.1 Sample identification

There are 4 separated frames for entering the sample identification. Using this, a separation in distinct columns within the result and export table is possible.

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

- ☞ The content of the first sample identification frame will be displayed in the Overview mode below each measuring position. If there are too many signs, the text will be shortened.

- ☞ To facilitate combined data searches in the MicroAssist evaluation program, a structured data entry is strongly recommended.
  i.e.: sample ID in Info field 1; Method in Info field 2, Manufacturer in Info field 3 etc.

3.4.6.2 Drift Balance 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).

3.4.6.2.1 DropStop

If this function has been activated in the “Measurement Parameters” menu (cf. Fig. 3.6), then the measurement signal is not allowed to show values less than zero.
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 (cf. Section 5.2.2). Thus the signal is reverted into its original

shape.

3.4.6.2.2 Drift Balance

Using that function a positive deviation of the measurement signal within the pre-set time scale is prohibited. It 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 Balance function, this can be corrected after the measurement has been finished during data evaluation (cf. Section 5.2.3). 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 3.2.1 to 3.2.6 can be stored in the database. They are then easily selectable in a single step for subsequent measurements.

To save analyses, select the menu option "Analysis/ Create new". After selection of “Create new” the “Analysis edit form” window will appear and the analysis parameters to be stored can be entered into the table.

A new set of fields for entering analysis parameters will appear after pressing the **“+”** radio button. New settings need to be confirmed by pressing the **“✓”** button. The **“x”** button will discard non confirmed settings and the **“-”** button can be used to remove the analysis highlighted.
The “ ▶ ”, “ ◀ ”, “ ▲ ”, “⏮” and “⏭” buttons are navigation buttons to navigate within already stored analyses.

Stored analyses can be selected in the Analysis area of the MicroTrac Measurement Parameters window. The selection has to be confirmed by selection of the blue **“↓”** button beside the analysis selection window.
When storing an analysis, a direct link to an already stored calibration is possible. To establish that link the precise name of the calibration must be entered within “CalName” during entering the parameter to be stored for the actual analysis.

Alternatively, the parameters set for an individual measurement position can be copied using the “copy all fields” = “Alt C” function in the “edit” mode of the “Measurement Parameter” menu and can be entered for other positions by selecting the “insert all fields” = “Alt V” function. After copying confirmation is necessary by selecting the red “✓” confirm button.

☞ Parameter settings that have been changed appear in a red colour and need to be confirmed by pressing the red confirmation button (cf Fig. 3.6). Then the changed parameters are definitely entered for the selected position and will be 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 **"enter"** for individual changes. Pressing **"Alt enter"** confirms all settings that have been changed (same function as the confirm button)

☞ By pressing the "waste" button all settings that have not been confirmed will be discarded resulting in a reset to the previous parameter settings.

☞ For a proper operation of the program when working with calibrations they should be linked to an analysis.

3.4.8 User Identification

Using the field "user identification" an analysis can be linked to a user. The user name can be selected using the scroll button on the right.

New users can be entered using "User administration" within "Extras" in the MicroTrac Overview form (cf. Fig. 3.6).

3.4.9 Extended copy functions for measurement parameter alignment

Extended copy functions for the simultaneous alignment of measurement parameters and sample descriptions to various positions within one incubator or for copying of all the parameters of an incubator to another incubator are available when selecting the function "Edit" within the MicroTrac Overview window.

Explanation of the copy functions available:

"Copy position parameter"
Using this function all parameters (sample description and measurement parameters) of a single position focused via selection with the mouse will be copied to a copy buffer . The copied position becomes visible via a red dotted ring moving around the position sign. This function is also accessible via the right mouse button "Copy position".

Selection of target positions
After a position has been copied, the target positions can be selected with via mouse-click while simultaneously pressing the "Shift" button, more than one consecutive position can be selected. If more than one target position will be selected they will be displayed with a blue ring.

"Insert info texts only"
Using this function only the sample descriptions (contents of Info fields 1 to 4) will be inserted into the selected target positions.
This function is also accessible via the right mouse button "Insert sample description".

"Insert all parameters"
Using this function all parameters (info texts and measurement parameters) will be inserted into the selected target positions.
This function is also accessible via the right mouse button.

IMPORTANT NOTE:
This function is only applicable for positions that are empty. If positions are not empty within your selection, they will not be considered for copying. For positions with ongoing measurements an error message will appear and the parameters are not pasted.

"Clear buffer"
This function will clear the copy buffer and will deselect the copied positions.

"Text edit table"
Using this function a window for simplified entering of sample descriptions will be opened.

☞ Positions without any information entered cannot be copied.

4 Performing tests

The measurement parameters (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 are filled immediately before use with the corresponding pre-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 1.5 ml of a freshly prepared (as far as possible) 0.2% KOH solution (0.2 g KOH platelets 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. KOH solution which has been stored for a longer period can be regenerated by heating for a short period a single time; but make sure that the concentration has not been altered as a result of water evaporation.

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

Filling of the polythene measuring cells with sterile nutrient must 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 *BioTrac* 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 threshold for the investigation of liquid samples is > 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.

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 10g sample is typically homogenised with 90 ml of a sterile ringer solution or sterile peptic water. For the *BioTrac* 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 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 from 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.

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 give an extra turn to the lids of the measuring cells which have already been tightly closed immediately before placing the cells into the *BioTrac* 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.

If lids with gaskets will be used for the indirect polystyrol measuring cells, additional tightening before insertion of the cells becomes no longer necessary and the lids can be also re-used for several times.

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 and glass inner container lids are loosened or completely removed before placing in the measuring cell, 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 *BioTrac* 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 not immediately removed from the incubator then, 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.

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 measurement parameters will be re-set to the factory defaults and must be selected again for any new measurement.

☞ It is possible to remove measuring cells for a short time span within 2 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.

5 Display and evaluation of measurement results

5.1 Display options and evaluation of measurement results using the MicroTrac measurement software

Use the function key F6 or double click with the mouse key slightly outside a circle icon within the “MicroTrac Overview” window to access the "MicroTrac Measurement parameters" window.
Use the function key F7 or double click within the circle icon in order to access the "MicroTrac Measurement mode" window for the curve selected and the neighbouring curves.
To revert to the MicroTrac Overview window, close the program window selected or use the function key F5.

5.1.1 MicroTrac Overview mode

The overview display shows the following information:

- Measurement duration / end of measurement (information)
- Sample description (Info field 1)
- Sample status (evaluation)

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" menu option).

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.

Sample status (evaluation)

If the traffic light evaluation system is in use (red/green or red/yellow/green, see 3.4.5.1), the ring around the circle icon in the overview display 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.

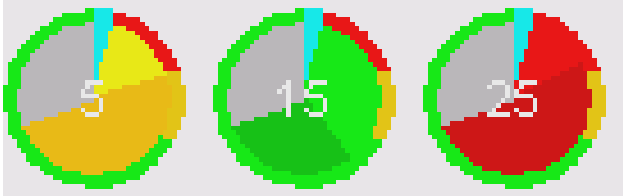
The progress of the measurement is still indicated by the dark blue-filled circle segments. If the measurement signal exceeds the pre-set threshold value, however, the entire part of the circle is immediately displayed in the colour corresponding to the sample evaluation (red or yellow).

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

This makes it easy to determine whether, for example, 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 precise end of the measurement cycle is indicated in the text window opened below the circle icon if the mouse pointer will be moved into that circle in which is given the time (for measurement cycles of less than 24 hours) and the date (for measurement cycles lasting longer than 24 hours).

Sample description

The sample description uses the contents of the first information field (sample description) from the “Measurement parameters” menu. The text appears below the circle diagram as described above and is abbreviated as required.

5.1.2 Individual view (measurement curve)

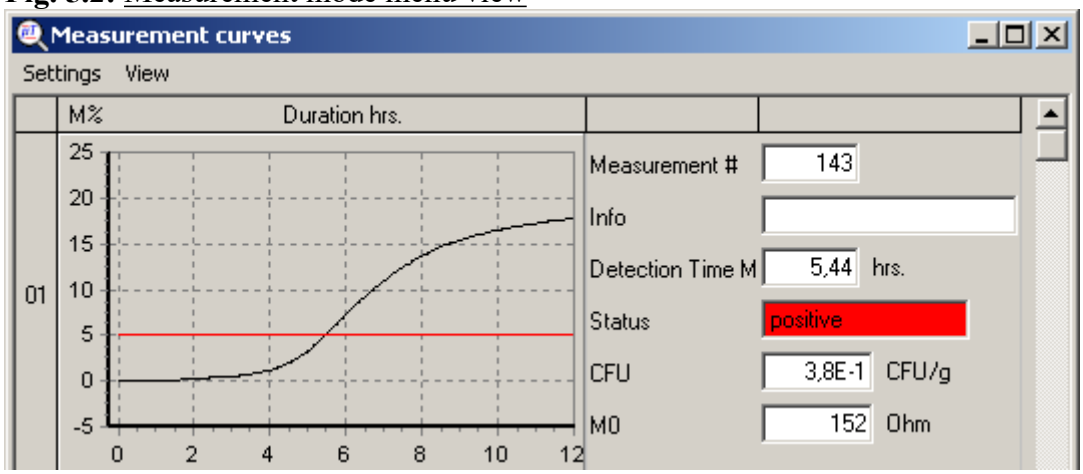
The “Measurement curves” menu allows a detailed view of the measurement curves based on the evaluation criteria for that position, as specified in the “Measurement parameters” menu.
Figure 4.2 shows a typical view of the measurement progress.

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”, “Status” and “CFU” are displayed along with the actual measurement curve.

The microbiological count corresponding to the detection time at the threshold set is only calculated and displayed, however, if a calibration was selected for that measurement.

Fig. 5.2: Measurement mode menu view



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

SCALE
The scale of the diagram can be adapted or changed as required under "Settings" in the "Diagram axis" menu option.

DISPLAY OF MEASUREMENT POINTS
Individual measurement points can be displayed by selecting "Display measurement points" in the "Settings" menu option.

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

QUANTITY OF DIAGRAMS AND DIAGRAMSIZE DISPLAYED
The quantity of the diagrams displayed is dependent of the screen size of your PC-Monitor. 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 reset to the original size by activation of the function “Settings/Reset diagram size”.

SCROLL
You can scroll between the current measurement positions using the arrow keys ↑ (to scroll upwards) or ↓ (to scroll downwards).
You can scroll up and down within individual data records using “Scroll ↑” or “Scroll ↓”.

5.2 Correction of measurement signals

The "Measurement parameters" menu has, as already described in 3.4.6.2, the option of correcting atypical measurement curves via the use of the “DropStop” and “Drift balance” functions. Corrections can be performed for ongoing measurements as long as they are still

active. For already finished measurements this can be done using the MicroAssist software just to view and/or print these changes. None of the changes are saved to the database.

5.2.1 Drift Balance

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 (if using calibration).

Since detection on the basis of the above phenomenon is incorrect, it is possible to reset the signal to the baseline within a pre-determined time period (= null value).

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

This will result in recalculation of the detection time and of the microbiological count (if calibration was used).

Since "Drift Balance" causes a massive interference of the measurement signal, the measurement curve for the selected period is no longer displayed in the measurement curve display.

☞ 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 Balance".

5.2.2 DropStop

In contrast to “Drift Balance”, “DropStop” prevents the signal from drifting into negative values.

This allows correction of negative drift, i.e. the short-term slide of the measurement signal into the negative range.
As this action does not significantly alter the detection time or the microbiological count calculations, at least in conventional impedance measurement (direct process), it is applied automatically for the duration of the measurement.

☞ The “DropStop” function has only a minor effect on detection times and microbiological counts.

The “DropStop” function is primarily used to render more commensurate individual measurements in cases where a few measurements have been subject to negative signal drift in direct comparison with other measurements.

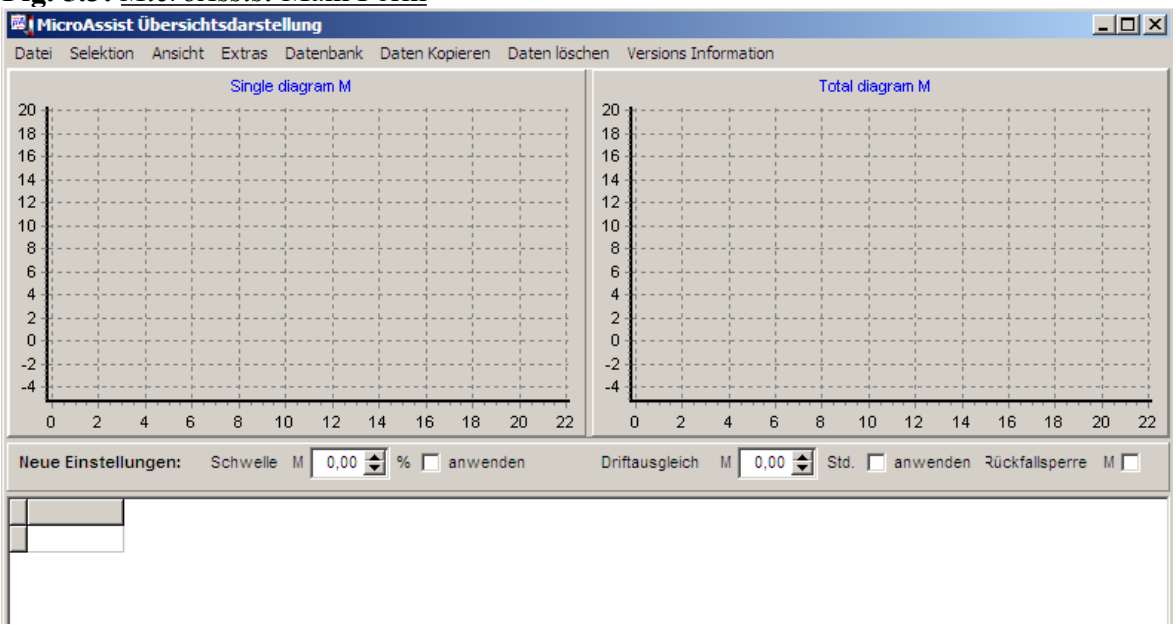
5.3 Data evaluation

A more detailed evaluation of measurement results, not only based on the simple options for monitoring measurement results using the MicroTrac measurement software is carried out using the *MicroAssist* program which can also be found in the *MicroTrac2* program directory.

5.3.1 Selecting measurement results

After opening the *MicroAssist* program you will access the program main form (see Fig. 5.3).

Fig. 5.3: *MicroAssist* Main Form



You can set the language in the "Extras" menu option as described under 3.1.7.1

Before you can start with the evaluation of data you have to select the measurements you want to display, evaluate print or to export the results for further processing.

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 descriptions and information entered in the Info fields
- Sample status
- Date of analysis (from to)
- Instrument serial No.

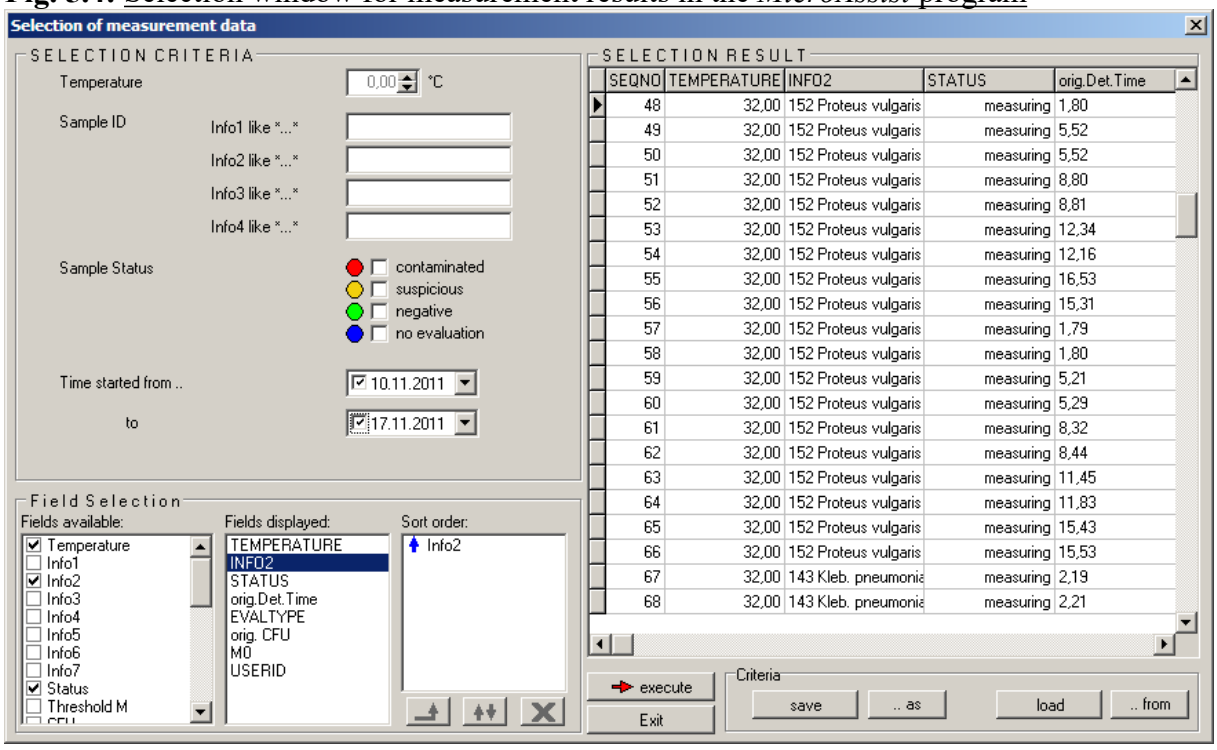
All the criteria selected for the search for measurement results are linked using the "*and*" function.

If you enter 37°C for the temperature and SYL* in the information field 1 and select "positive" for the evaluation, then activate the "Execute" command, the program carries out a search for those samples whose incubation temperature was 37°C, whose sample description starts with "SYL" and which are evaluated "Positive".
For a text search within the info fields the following options are available:

- Full text
  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.

Fig. 5.4: Selection window for measurement results in the *MicroAssist* program



To customise your results table use the "Field Selection" function to select from the "Fields available" 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.
Additionally the priority for sorting the selected fields can be defined in the window “Sort order”.

The customised selection criteria can be stored on demand using the functions “save” or “save ..as”. Files of the type xxx.CND will be generated directly within your MicroTrac2 program directory. Using “load” or “load ..from” stored criteria can be loaded for future selections using identical search criteria.

After entering your search criteria and selection of the fields to be displayed, start the search by pressing the “execute” button.

Then close the selection window and revert to the *MicroAssist* Main Form.

5.3.2 Display of measurement results

The selected data are integrated immediately after activation of the “execute” button into the MicroAssist Main Form window (Fig.5.3) and can be displayed as any combination of individual diagrams and/ or total diagrams and value tables.

The display mode for the various diagram types is selected using the “View” function.

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

To change the size within the left- and right diagram window place the cursor (pointer) of the mouse precisely between the two diagrams until the pointer changes into a left and right arrow, press the left mouse button and move the mouse towards the diagram you want to reduce.

For the differentiation of measurement curves within the total diagram window the colours must be selected and aligned using the ”Color selection” function within “Extras/ Diagram” (for details see Fig. 5.5).
Altered colour settings are saved as new default.

The colours selected will not only appear in the total diagram mode but also as a discrete colour flag left of the measurement number in the display table (cf. Fig. 5.6).

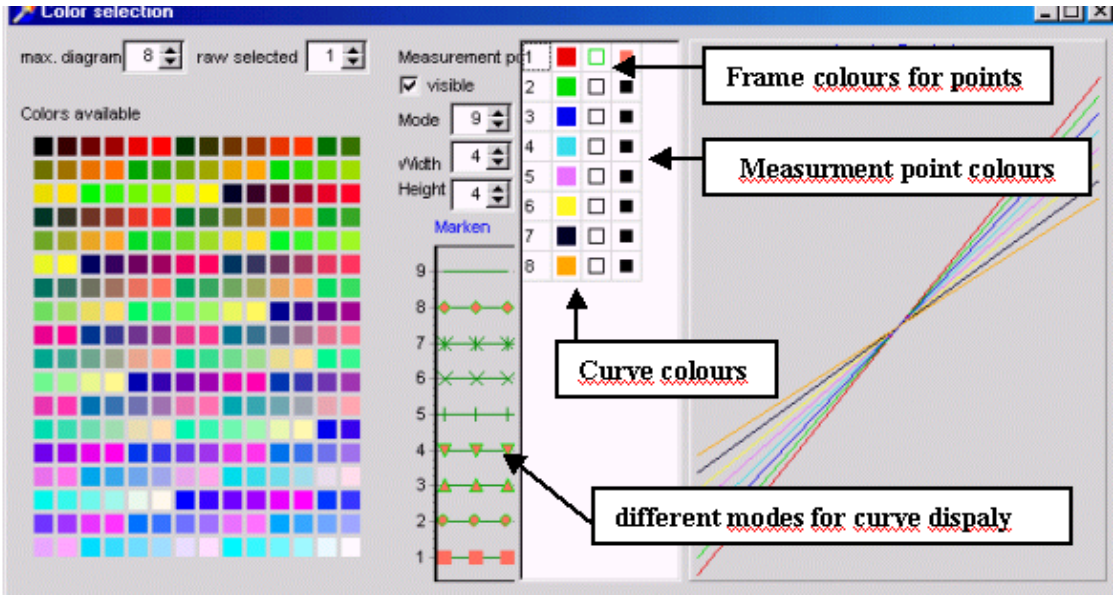
After selection of the max. number of diagrams to be shown in the total diagram window, the available colours can be aligned either for the total curve (first sign in the raw) or just for marking the outer frame of the signs for the measurement points (second sign in the raw) or to fill the signs for the individual measurement points with a colour selected (third sign in the raw).

In addition 9 different modes for displaying the curves can be selected.

Width and height of the different display modes 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.

Fig. 5.5: Colour selection



You can select a new threshold value for the selected data record by selecting "new settings: Threshold M". The detection times and CFU´s (if a calibration has been used) are recalculated automatically and are shown in red. The new detection time for M is displayed within the “new det.time M” 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.

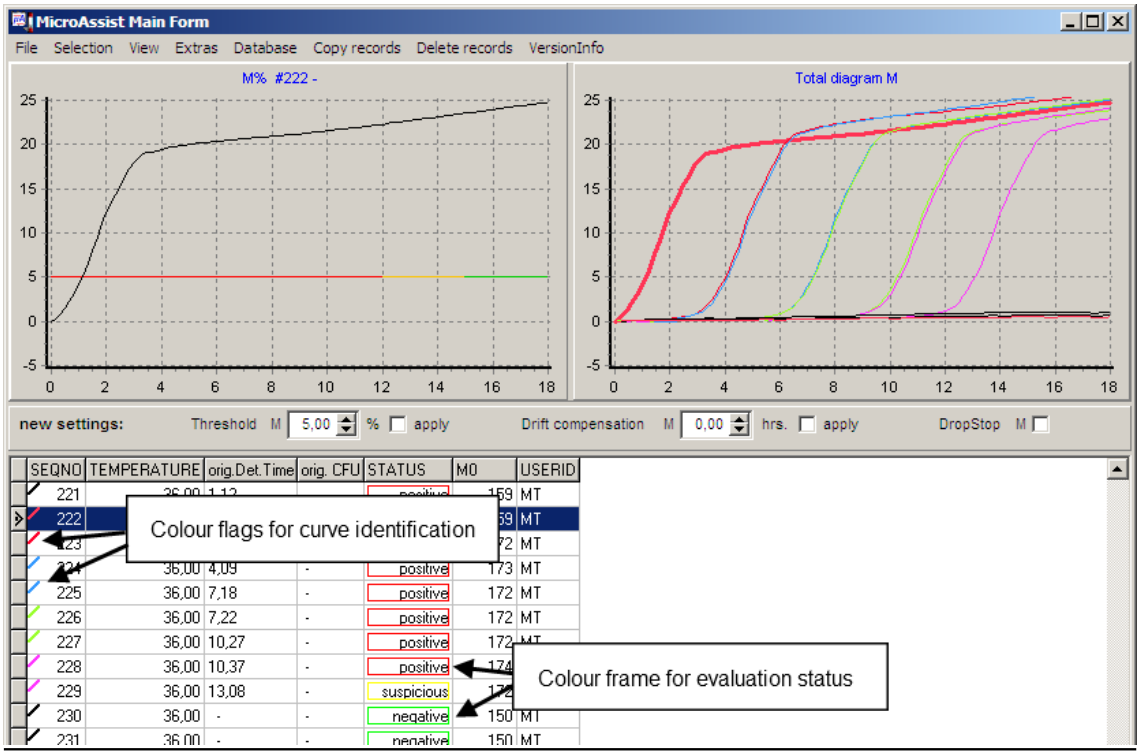
- ☞ The detection time displayed within the column named “orig.Det.Time” represents the detection time that has been registered during the original measurement and which is stored in the database. If no evaluation has been selected for the measurement no detection time is stored in the database and the value remains “-”. 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.

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

Please note that the sample status (red/yellow/green/blue) is also 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.

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 diagram
- Total diagrams
- Table

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

☞ For printing on black/white printers the “Print Black/ White only” checkbox must be activated.

☞ The printout of the table contains only as many columns as the sheet size allows.

5.3.4 Exporting data

The “Export selection table” function under “File” is used to export the contents of the result table as a text file with columns separated by "tabs" to a directory specified by the user.
The tabulated results can then be imported into any supported data processing program (e.g. MS Word, MS Excel...) and processed further.
The “Export selection for MS_Excel” function under “File” is used to export the measurement data of selected curves as a text file with columns separated by “tabs” to a directory specified by the user.

The tabulated results can then be imported into data processing programs and processed further.
Statistical evaluations are therefore very easy to perform, e.g. using MS Excel statistical functions.

Furthermore selected data can be exported by the function “Export selected measurements to database” which then can be transferred to a different PC and imported into the existing database there 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) database with MicroAssist..
The database that is currently activated = processed database, is displayed in the status bar of MicroAssist under the “database” tab .
The primary database (= operating database) always remains open. It is possible to switch between the two opened databases.

NOTE:
Only one additional database can only be opened at the same time besides the primary database. If a secondary database is opened another cannot be opened until the first one is closed again.

Opening a secondary database:
Is done via menu point “File” / “Open MicroTrac Database (new format)”. A window is displayed where the list on the right displays all ODBC database links 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!”**. (MicroTrac 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.

Switch between both databases:

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

Closing the secondary database:

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

6 Calibration and setting of evaluation criteria

The timing of the generation of the measurement signal is used as a measure of the microbial contamination of the sample under investigation.
The earlier the pre-set time 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 detected responds in an indirectly proportional manner 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 ensuring early indication of exceeded limits values.

The proportionality of the germ count of a sample in relation to the impedance detection time, taking the impedance detection time (x axis) and the decimal logarithm of the germ count determined using the reference method (y axis) in an x-y diagram is usually largely linear (10^3 - 10^7 CFU/g or ml).

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

The most essential criteria 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 and influential values for establishing a calibration

While 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), in the 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

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.
It is principally possible to use different starting materials, but this requires special consideration during establishment of the regression curve.

If it emerges 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 is not the same for all 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 from the point of view of 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

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

For proliferation to occur in nutrient media during the biological analysis therefore requires a longer adjustment phase for bacteria in a product with a low water-content.

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 as an example the investigation of milk powder and of pasteurised milk:

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.

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 *BioTrac*, as well as in the reference processes are carried out at the same temperatures.

As already thermally processed samples in the impedance analysis give essentially reproducible results at an incubation temperature of 37°C in comparison with 30°C (reference process), it is sometimes also necessary to carry out impedance processes and reference processes at different temperatures.

It is important, however, that the temperatures *per process* are 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 dependant upon volume.

Although the relative measurement used in the *BioTrac* makes the process more robust in this respect, take care nevertheless to use constant nutrient media and sample volumes.

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 value pairs 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 4 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.

In the vicinity of the production environment the majority of samples have very similar germ counts. In this kind of situation we recommend methods (below) for increasing/reducing the germ counts of products.

On no account must a product be artificially contaminated with microorganism cultures grown up freshly by separate enrichment (overnight cultures) for the purposes of increasing the germ count.

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

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). It should be noted, however, that the product itself can influence the growth of microorganisms and that this influence must not be changed excessively 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 progressive 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 on 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 in 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 (section 6.2) artificial contamination using freshly grown microorganism isolates is to be avoided as this can greatly interfere with 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 if it is allowed to adjust 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).

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 investigate sample dilutions for the impedance process.

If it is necessary to prepare sample differently, then take care to ensure the homogeneity of starting materials. The process for the sample preparation is as 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 *BioTrac* 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 *BioTrac* measuring cells using sample homogenates
- Placing measuring cells into the *BioTrac*
- Inoculation of plates for the reference process

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

6.3.4 Establishment and calculation of calibration curves

All measurements carried out for the production of the calibration are selected using the MicroAssist program and then are exported using the “export files table” function.

It is recommended that the selected data are 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 detection-threshold value.

The calculation of the regression equation and the graphical display of the regression lines is carried out via the accompanying Excel spreadsheet entitled "Calibrat.xlt".

If the exported file is opened in MS Excel, the sample descriptions and associated file numbers and impedance detection times (for each threshold) are inserted via the import function of MS Excel.
The CFU values obtained via the reference method in parallel are then entered in the germ count field.

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 indicated.

The nearer the correlation coefficient r 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 process and the reference process.

A correlation coefficient that is close to the ideal value has no value on its own, though, in terms of evaluating 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.

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 especially carefully (see 6.5.1).

☞ 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 actually provide an exemplary selection of a range of options. Other suitable statistical methods are also admissible.

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 α=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, α = 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 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 6 but ideally 10 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

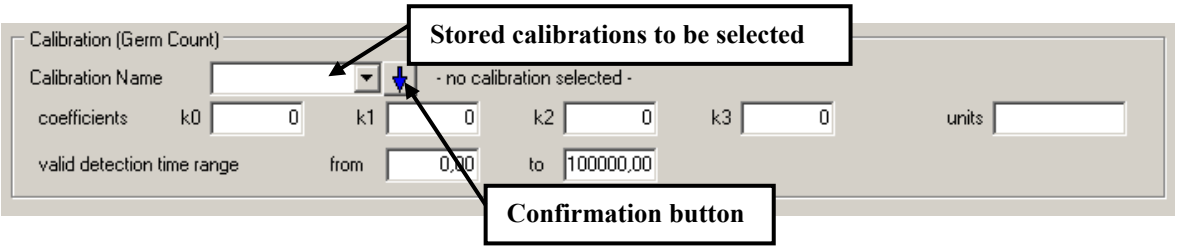
6.6.1 Saving and selecting calibrations in the MicroTrac software

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 (*BioTrac*) and CFU (reference method) is described using the following equation type:

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

After entering both coefficients k0 and k1 in the field provided for the entry of calibration parameters in the “MicroTrac Measurement parameters” window
(see fig. below) 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 “unit” input field.

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



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

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:

The equation for a linear regression is as follows:

$y = -0.432\ x + 7.43$
in which:
k0 = 7.43
k1 = -0.432

The equation for a quadratic regression is as follows:

$y = 0.021\ x^2 - 0.67\ x + 13.72$
in which:
k0 = 13.72
k1 = -0.67
k2 = 0.021

Additionally, 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 calibrations select the menu option "Calibration / Create new". After selection of "Create new" the "Calibration edit form" window will appear and the calibration parameters to be stored can be entered into the table.
A new set of fields for entering calibration parameters will appear after pressing the "+" radio button. New settings need to be confirmed by pressing the "✓" button. The "**x**" button will discard non confirmed settings and the "-" button can be used to remove the calibration highlighted.

The “▶”, “◀”, “▲”, “⏮” and “⏭” buttons are navigation buttons to navigate within already stored calibrations.

Stored calibrations can be selected as shown in Fig. 6.1 within the calibration part of the measurement parameter window. The selection has to be confirmed by selection of the blue “↓” button (see Fig. 6.1)

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

☞ ***For working with calibrations it is extremely important that the threshold (type and threshold value) that has been used to establish the calibration will be used.***

☞ ***For a proper operation of the software a calibration should be linked to an analysis.***

6.6.2 Setting evaluation criteria (sample qualification)

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

The criteria described in 3.3.5 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 5000 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 of < t = 6.1 hours thereby exceed the threshold

value and must be rejected accordingly.

Classification criteria are summarised as follows:

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 3.4.1) to be entered in the measurement parameters menu option should 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 only one value is available, i.e. samples are classified once that value is exceeded, only that one value is entered into the calibration equation.

Only the time for **red** is entered in the "measurement parameters" menu option. 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 menu option.

Any sample that exceeds the set M-threshold within the measurement period is then 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).

It is 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.

On that basis 2 time limits are determined:

> **Time limit 1 (red**) represents that time which, if not exceeded, indicates that the germ count in a sample under investigation highly probably falls above a specified threshold value.
>
> **Time limit 2 (yellow)** defines the range in which the probability of the threshold value being exceeded is present, but within which a result just on or below the threshold value cannot be excluded (= uncertainty range or grey zone).
>
> All samples which do not exceed time limit 2 are classified as **green,** indicating a germ count below the threshold value (minimum one log stage)

In contrast to producing 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 6.3).

The results are summarised in 2 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 then 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. (7 hours in above example).

Time limit 1 (red) is the time within which the majority of positive (above the threshold limit) samples are detected (5 hours in above example).
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 re-measured or re-clarified with the reference process.

Example:

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

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

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

	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	>13 hours
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) (see tables 6.1. and tab. 6.2).

The time for the **warning value** (the time within which all samples above the threshold lie = **yellow**) is easy to read off (7 hours in above example)

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.

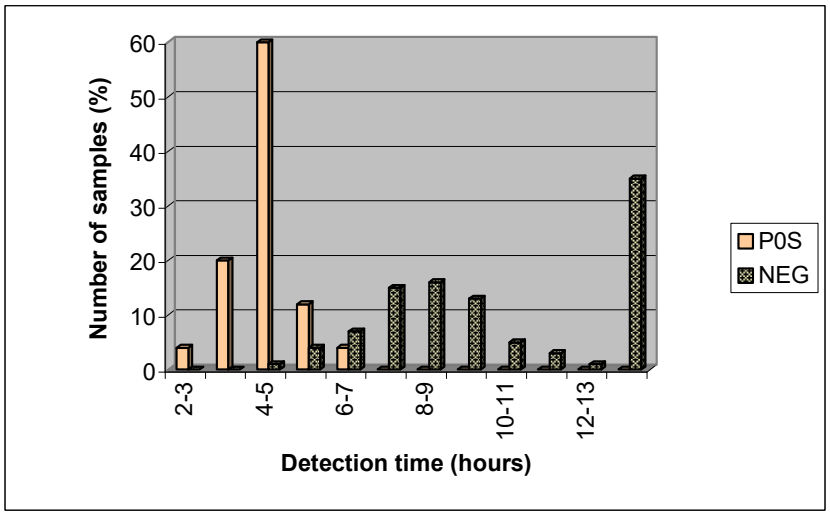
At a value of 7 hours for the warning value (= time limit 2) in the above example, 96% of samples above the threshold value are correctly classified), 1% of samples below the threshold value are incorrectly classified as being positive. In total, 96% of samples giving results above the cut-off value of 5 hours are correctly classified as clearly above specification (red).
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% (see table 6.3).

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*

As table 6.3 shows, 16% of samples with results above the threshold value and 11% samples with results below the threshold value fall within detection times of between 5 and 7 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.

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



7 Further investigations and confirmatory reactions

After a *BioTrac* measurement has been finished, the contents of the measuring cells (the enriched cultures) are available for additional investigations if wanted.

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.

BiMedia 660A (selective media for the detection of Clostridia) contains an indicator system for the detection of H_2S- production from Thiosulfate by blackening of the media.

Addition of chromogenic and/ or fluorogenic substrates (i.e. MUG) to the growth media is additionally possible in many cases.

7.2.2 Indole test

Using BiMedia 160C the classical indole-reaction (indole production from tryptophane) can be performed after addition of 1-2 ml of Kovac´s indole reagent directly to the measurement cell for reactive samples.
In the case of presence of *E. coli* a purple-red ring is formed on the surface of the media after only a few minutes.
The indole reaction can be used as an absence criteria 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 or molecular biological methods as confirmatory assay of reactive samples has been proved valuable.

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 reliable.

Most of those methods can be used directly with the enrichment culture present in and out of the measuring cell.
Salmonella enrichments with positive impedance signals can be therefore confirmed within minutes by using immunochromatographic rapid tests.

As most of the immunological reactions are convincing by there rapidity, molecular biological confirmatory reactions show an exceptional specificity.

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 therefore facilitates a variety of investigation options, which go far beyond the normal analytical range of nutrient media.
At SY-LAB therefore, every batch is investigated, with precisely defined test strains for characteristics impedance parameters such as *impedance detection time (IDT) and formation of growth signal*. Only after thorough comparison of these parameters with the corresponding specifications and with the results of a similarly investigated batch, is the batch released for sale or rejected.

The BiMedia product line from SY-LAB undergoes continuous outstanding quality control which actually allows us to guarantee its impedance-specific performance.

These media, which have been developed specifically for use in impedance analysis, are invaluable, therefore, for routine investigations. They ensure the corresponding performance of the media.

If using standard formulations or media developed in-house, however, these should be correspondingly validated against a reference process.

9 Troubleshooting

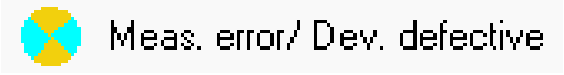
9.1 Error messages MicroTrac

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 balance the signal can be corrected if there was an influence on detection time.

PROBLEM
The data received from the *BioTrac* 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.
If the error message will continue for the following measurements an instrument failure must be considered.



PROBLEM
No measurement data have been received from the instrument.

SOLUTION
Either the respective incubator poeitin or the whole *BioTrac* device have an electrical error / are defective. Please call Service Personel.

PROBLEM
An error other than those specified above ocurred.

SOLUTION
Please call Service Personel 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 set temperature.

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 Service Personel if this error message appears frequently.

“Ungültige Variant. Typumwandlung”

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

SOLUTION
Select an analysis and confirm by activation of the confirmation button or confirm without selecting an analysis

The measurement parameter window cannot be closed.

PROBLEM
No user ID has been selected

SOLUTION
Select a user and confirm.

Another program language appears on start up

PROBLEM
The language selection has not been saved.

SOLUTION
Select the desired language again and save your settings using by activation of „save Settings“

“Unable to connect to the database...“

PROBLEM
The directory “Database” has been renamed or removed from the MicroTrac program directory.

SOLUTION
Check name and/or location of the “Database” folder.

The function “Determine device” within the installation menu is not accessible or causes an error message “buffer not empty”

PROBLEM
The COM-Port buffers have not been emptied properly by WINDOWS

SOLUTION
Close the installation menu and start the instruments. During the instruments start up the COM-port buffers will be emptied.
The status indicator will remain RED for a prolonged period of time.

9.2 Device temperature

The actual 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 to a cooling device.

The actual incubator temperature is significantly *lower* than the nominal temperature

PROBLEM
The heating system of the incubator is defective.

SOLUTION
Please call service personnel.

The nominal temperature of an instrument with active cooling cannot/ can hardly be reached	PROBLEM The flow through of the cooling liquid is too high or the temperature of the cooling liquid is too low **SOLUTION** **Reduce the flow our increase the temperature of the cooling liquid.**

9.3 MicroAssist errors

No files found after selection	Please check the selection parameters and the date.
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 MicroTrac program directory. **SOLUTION** **Check name and/or location of the “Database” folder.**

10 Index

NOTES: