BactoReal® Kit
P. aeruginosa & Enterobacter spp.

Instructions for Use

CE

IVD **For *in vitro* diagnostic use**

REF **DHUS00553**

Σ **50 reactions**

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Explanation of symbols

Symbol	Meaning	Symbol	Meaning
LOT	Batch code		Use by
REF	Catalogue number		Manufacturer
Σ	Contains sufficient for <n> tests		Store at
CE	This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices	IVD	For *in-vitro* diagnostic use
	Consult instructions for use		Manufacturer date
	Corrosion, GHS05		Exclamation mark, GHS07

1. Intended use

BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. is a non-automatic *in-vitro* diagnostic real-time PCR test for the qualitative detection and identification of extracted DNA of *Pseudomonas aeruginosa* (16S rRNA gene) and *Enterobacter* spp. (rpoB gene) from samples purified from human EDTA blood, CSF aspirates and biopsies.
In combination with other diagnostic tests such as culture it supports a rapid and specific diagnosis for patients suspected of having a bacterial infection. Results have to be interpreted in context of the overall picture and other clinical parameters. Sepsis diagnosis must not be based solely on BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp..

2. Product description

BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. detects the 16S rRNA gene of *P. aeruginosa* and the rpoB gene of *Enterobacter* spp.

A probe-specific amplification curve in the fluorescence channel for FAM or VIC detects the pathogen-specific DNA. The Internal Positive Control (IPC) is detected in the fluorescence channel Cy5 and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for the DNA IPC is added during sample extraction.

This test has been validated with the Applied Biosystems® 7500 Real-time PCR System (Thermo Fisher Scientific) and with the cobas z 480 Analyzer (Roche). However, it is also suitable for other real-time PCR instruments that can measure and differentiate fluorescence in the FAM, VIC and Cy5 channel (e.g. LightCycler® 480 II).
This test is based on real-time PCR. A specific DNA sequence of the pathogen genome is detected and amplified. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplificates.

Further tests for diagnosis of sepsis, which can be combined with BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp:
BactoReal® Kit *E. coli* & *Klebsiella*, order no.: DHUS00153
BactoReal® Kit *Staphylococcus* spp. & *S. aureus*, order no.: DHUS00253
BactoReal® Kit *S. pneumoniae* & *Streptococcus* spp., order no.: DHUS00353
BactoReal® Kit vanA & *Enterococcus* spp., order no.: DHUS00453
BactoReal® Kit *A. baumannii* & *P. mirabilis*, order no.: DHUS00653
MycoReal Kit *Candida* & *A. fumigatus*, order no.: DHUF00153

3. Background information

Pseudomonas spp. are Gram-negative rod bacteria found in soil, water and plants. In healthy individuals, they do not cause infection, but immunocompromised people can be infected. *Pseudomonas aeruginosa* as well as other *Pseudomonas* species such as *P. paucimobilis, P. putida, P. fluorescens* or *P. acidovorans* cause nosocomial and antibiotic-resistant infections which are difficult to treat and can be life-threatening.
Pseudomonas infections can lead to sepsis, with *P. aeruginosa* being the most common species. *Pseudomonas* bacteremia is indicative of contaminated infusion solutions, medications or disinfectants used in the placement of intravenous catheters.
P. aeruginosa is also responsible for wound infection and bacteremia in burn patients, with high rates of multidrug resistance.
Enterobacter species are Gram-negative bacteria causing a wide variety of nosocomial infections, including those affecting the lungs, urinary tract, intrabdominal cavity and intravascular devices.
E. asburiae can be detected in urine, stool and blood samples. *E. bugandensis* can cause neonatal sepsis. *E. cloacae* can lead to wound infections and catheter-associated urinary tract infections and is often resistant to antibiotics. *E. ludwigii* and *E. roggenkampii* belong to the *Enterobacter cloacae* complex.

E. huaxiensis, *E. chuandaensis* and *E. chengduensis* were discovered in the last few years in China and can cause sepsis. *E. oligotrophica* is an oligotrophic bacterium and has been recently isolated in Japan. *E. sichuanensis* was isolated in 2016 from a urine sample taken from a patient in China.

4. Principle of real-time PCR

BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. is based on multiplex real-time PCR technology by 5´-nuclease-assay technology. Specific DNA sequences in the pathogen genome are amplified and the generated PCR-products are detected by oligonucleotide-probes labelled with fluorescent dyes (VIC, FAM). This allows a sequence-specific detection of PCR amplificates.
During PCR, primers are extended by *Taq* polymerase and probes hybridized to the target are cleaved by the 5'-exonuclease activity of *Taq* polymerase. According to the accumulation of PCR product, the fluorescence of the probe increases with each PCR cycle. The change in fluorescence of the dyes is recorded cycle by cycle in the real-time PCR instrument in the closed reaction tube at different fluorescence wavelengths.
The Ct value (Cycle threshold, Ct = Quantification cycle, Cq = Crossing point, Cp) describes the cycle at which the fluorescence first rises significantly above the background fluorescence.

4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
Module 5 Assay Mix (green cap)	Primer and probe for - *Pseudomonas aeruginosa* (FAM) - *Enterobacter* spp. (VIC) - DNA IPC (Cy5)	1 x 50 µl	-15°C to -25°C
DNA IPC Target (orange cap)	Target for DNA IPC (internal DNA positive control system)	1 x 100 µl	-15°C to -25°C
Module 5 Positive Control (red cap)	DNA positive control (approx. 1,000 target copies/µl)	1 x 200 µl	-15°C to -25°C
DNA Reaction Mix (white cap)	DNA reaction mix*	1 x 500 µl	**-15°C bis -25°C, until first use, then +4°C**
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-15°C to -25°C

* **DNA Reaction Mix:** The Master Mix provided with the kit has been designed for reliable, high-sensitivity real-time PCR. The Master Mix contains a highly purified *Taq* Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle RT-PCR inhibitors.

The components of BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. are stable until the expiry date stated on the label and are delivered at +4°C.

5. Additionally required materials and devices

- Suitable reagents and laboratory equipment for DNA extraction of bacteria from one ml of blood at least, or aspirates, CSF and biopsies.
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM, VIC, Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes with optical closing material recommended by the manufacturer of the real-time PCR instrument
- Pipettes
- Nuclease-free water
- Pipette tips with filter

- Laminar flow box
- PCR workstation
- DNA decontamination reagent

6. Precautions and safety information

6.1. General information

- For *in vitro* diagnostic use. The use of this kit is limited to qualified personnel instructed in the procedures of real-time PCR and in vitro diagnostics.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Quality of DNA has a profound impact on the test performance. Ensure that the used DNA extraction system is compatible with real-time PCR technology.
- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light.
- The real-time PCR instrument should be serviced and cleaned regularly.
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste disposal.
- Please note the expiration date of the kit.
- **Caution:** DNA IPC target is stored in RNA/DNA stabilizer which contains DTT/guanidine thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).

6.2. Specific information

A workflow must be followed to avoid false positive results due to detection of contaminating DNA from environmental pathogens.

Recommended measures to avoid DNA contamination:

- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Ensure that samples with high bacterial concentrations (e.g., faeces, saliva) are not processed at working areas.
- Laboratory benches and supplies must be cleaned regularly.
- Sample preparation should be performed in a laminar flow box. Clean laminar flow box regularly in all areas.
- Preparation of real-time PCR should be done in a PCR workstation.
- The use of sterile aerosol-resistant pipette tips is required.
- Use only DNA-free consumables.
- If possible, leave consumables and pipettes in the laminar flow sterile bench and PCR workstation.
- Wear lab coat.
- Work only with powder-free disposable gloves, do not touch the palm and fingers of the gloves on the outside when putting them on. Change gloves frequently. To avoid skin contact, wear gloves over the sleeves of the lab coat. Use disposable sleeve protectors if possible.
- Do not touch the rim or threads open vials.
- Care must be taken when handling specimens and positive control to avoid cross-contamination.
- Storage of positive and potentially positive material should be separate from all other reagents.
- For a valid interpretation of results, a negative control shall be included during DNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with DNA of pathogen during extraction.

7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate DNA extraction procedure.
- This kit has been used to validate the detection of bacterial DNA from EDTA blood, aspirates, CSF and biopsies. If the concentration of leukocytes in the blood is low, pathogen DNA may be lost during extraction.
- A negative test result does not exclude the possibility of infection with pathogenic organisms.
- Sequence variabilities in the target-region of some subtypes (strains) may lead to false-negative or less sensitive results.
- A pathogen concentration in the sample below the detection limit can lead to a false-negative result.
- A cut-off must be established for both *P. aeruginosa* and *Enterobacter* to avoid false-positive data due to contamination, which reduces the sensitivity of the test.
- Sepsis diagnosis must not be based solely on BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp.. The test has to be used in combination with other diagnostic tests such as culture to enable a rapid and specific diagnosis for patients suspected of having sepsis or a bacterial infection of the bloodstream.
- Results should be interpreted in the context of clinical and laboratory findings.

8. Preparation of samples

Ensure that the extraction system used is not contaminated with DNA of pathogens detected with BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp.. Extract the sample using a DNA extraction system compatible with real-time PCR technology.

Sample preparation should be performed with the recommended measures to avoid DNA contamination (see point 6.2). Always include a DNA extraction negative control (NTC, e.g. extraction of water).

EDTA-blood: The extracted DNA analyzed per module should be equivalent to 50 µl of blood at least (e.g., 500 µl of blood eluted in 100 µl). If the sample is planned to be further screened for other sepsis pathogens, extraction of 1 ml EDTA blood in combination with mechanical lysis by beads is recommended.

The **DNA IPC target** has to be added during extraction. The DNA IPC serves as a control of the extraction, identifies possible PCR inhibition and ensures the integrity of the kit reagents.
Caution: The DNA IPC target must not be pipetted directly to the sample material, but must be added to the lysis buffer.
→ Add 1 µl DNA IPC target per sample to the lysis buffer.

Recommended automatic extraction methods for EDTA-blood:

- Optional: mechanical lysis of 1.3 ml EDTA blood with beads (e.g. MP Biomedicals™ Lysing Matrix E, 2 ml, mpbio) on the Magnalyser or another beadbeater (70 sec, 7000 rpm).
- Subsequently, automated extraction of supernatant (approx. 800 µl) using one of the following extraction kits:
  - innuPREP AniPath DNA RNA - KFFLX Kit (Analytik Jena) with a modified protocol for 800 µl sample volume using the KingFisher FLEX extraction device (Thermo Fisher Scientific).
  - MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific). Do not use the lyticase and lysozyme included in the kit and replace Proteinase K with Proteinase K, recombinant, PCR Grade (Roche) to avoid contamination.

9. Preparation of real-time PCR

- The preparation of the real-time PCR should be done in a PCR workstation (see point 6.2).
- Include one positive control and one extraction negative control (NTC) per PCR run and analysis
- Thaw kit components completely at room temperature. When thawed, mix components carefully, centrifuge briefly. Gently Mix the DNA Reaction Mix to ensure homogeneity of solution.
- **Positive Control**
  → Use 9 µl of Positive Control. Always add Positive Control last.

9.1. Pipetting scheme

		Per sample and module
Preparation Master Mix per module (mix well)	DNA Reaction Mix	10.0 µl
	Module Assay Mix 5	1.0 µl
	Module Master Mix	**11.0 µl**
PCR-reaction	Module Master Mix	11.0 µl
	DNA-Probe	9.0 µl
	Total volume	**20.0 µl**

→ **If DNA IPC Target was not added during extraction (not recommended):** Freshly dilute the DNA IPC Target 1:100 with nuclease-free water and add 1 µl per sample directly to the master mix. **Caution:** The use of more than 1 µl diluted (1:100) DNA IPC Target per reaction causes inhibition of the real-time PCR reaction.

For preparation of real-time PCR, dispense 11 µl aliquots of prepared Master Mix into the plate wells and then add 9 µl of DNA sample per well. At last, pipet the Positive Control.

9.2. Programming of temperature profile

Please find further information on programming of the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing a multiplex-PCR.

Detection channels: FAM-NONE, VIC-NONE, Cy5-BHQ1
Passive reference dye, if needed (depends on the device): ROX (e.g. ABI® 7500, QuantStudio™ 3/5/6/7)
Sample volume: 20 µl

Detection channels for cobas z 480 Analyzer (Roche)
FAM: Excitation at 465 nm, Emission at 510 nm
VIC: Excitation at 540 nm, Emission at 580 nm
Cy5: Excitation at 610 nm, Emission at 670 nm
No passive reference dye needed

Detection channels for LightCycler® 480 II (Roche):
FAM: Excitation at 465 nm, Emission at 510 nm
VIC: Excitation at 533 nm, Emission at 580 nm
Cy5: Excitation at 618 nm, Emission at 660 nm
After analysis of Cy5 channel, a colour compensation for FAM and VIC has to be selected from the Roche database.
Detection format: 3 Color Hydrolysis Probe, no passive refence dye needed.

Temperature Profile:

Program 1	Program 2	Program 3
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°C
50°C 2 min	95°C 20 sec	95°C 5 sec 60°C 30 sec

For ABI® 7500, QuantStudio™ 3/5/6/7:
Ramp speed: "Standard"
Without "fast cycling" parameter

10. Interpretation of PCR-data

Important: Samples should be analyzed in both logarithmic (Roche instrument: Abs Quant/Fit Points) and linear view and compared to the negative control. Please note that some PCR platforms require Color Compensation when using a multiplex PCR. When using LightCycler® 480 II, after analysis of Cy5 channel, a colour compensation for FAM and VIC has to be selected from the Roche database.

For the analysis of the PCR results, select the fluorescence display options FAM and VIC channel for the pathogen target and Cy5 channel for the DNA IPC Target in the logarithmic analysis. In addition to the Cq values (Quantification cycle (Cq) = Cycle threshold (Ct) = Crossing point (Cp)), also check the amplification curves and adjust the Threshold (noise band) in the logarithmic view if necessary. After saving the new settings, export the data. Export the tables per dye if working with a cobas z 480 Analyzer.

Low concentrations of pathogen DNA can often be present in the PCR and extraction reagents. Therefore, Cq values of the samples in the FAM and VIC channel must be interpreted in comparison to Cq values of the negative control of DNA extraction (NTC) also (see 10.1, 10.2).
Contamination with pathogen DNA is possible during sampling, extraction and PCR. Therefore, a cut-off with Cq=35 must be defined for both *P. aeruginosa* and *Enterobacter*. The sensitivity of the test (limit of detection, LoD) is reduced accordingly, see Table 3.

Table 1 shows the criteria for valid controls and **Fehler! Verweisquelle konnte nicht gefunden werden.** show interpretation of data with clinical samples.

10.1. Controls

Table 1: Criteria for valid controls, IPC Target was added during extraction.

	Cq FAM channel *P. aeruginosa*	Cq VIC channel *Enterobacter*	Cq Cy5 channel DNA IPC[1]	Interpretation	Action
Positive control	<30	<30	Negative	Valid	-
Positive control	Negative	Negative	Negative	Invalid	See 11.1
Positive control	<30	Negative	Negative	Invalid	See 11.1
Positive control	Negative	<30	Negative	Invalid	See 11.1
NTC[2]	Cq > 35	Cq > 35	28-32	Valid	-
NTC[2]	Cq > 35	Cq > 35	Negative	Invalid	See 11.1
NTC[2]	Cq < 35	Cq < 35	28-32	Invalid	See 11.3
NTC[2]	Cq < 35	Cq > 35	28-32	Invalid	See 11.3
NTC[2]	Cq > 35	Cq < 35	28-32	Invalid	Siehe 11.3
Negative control[3]	Cq > 35	Cq > 35	Negative	Valid	-
Negative control[3]	Cq < 35	Cq < 35	Negative	Invalid	See 11.3
Negative control[3]	Cq > 35	Cq < 35	Negative	Invalid	See 11.3
Negative control[3]	Cq < 35	Cq > 35	Negative	Invalid	See 11.3

[1] If the IPC Target has been added directly to the Master Mix, all samples must be positive in Cy5 channel
[2] Negative control of the extraction. Cut-off for *P. aeruginosa* and *Enterobacter*: Cq=35.0

[3] Optional

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid. If results of controls are not valid, the patient results cannot be interpreted.

10.2. Clinical samples

The results of the NTC as well as the cut-off must be included in the interpretation, see Table 2.

For *P. aeruginosa* and *Enterobacter* a Cq-value cut-off has to be defined. The Cq value for the cut-off is 35.0 (corresponds to approx. 50 target copies). Samples with Cq values lower than 35.0 are considered positive results, while Cq values greater than 35.0 may represent contamination and should be considered negative results.

Table 2 Interpretation of clinical samples

	Cq FAM channel ***P. aeruginosa***	**Cq VIC channel** ***Enterobacter***	**Cq Cy5 channel DNA IPC Target**	**Interpretation (see also Table 5)**	**Action**
Clinical sample	Cq > 35	Cq > 35	28-32[1]	Negative	-
Clinical sample	Cq < 35	Cq < 35	Positive/Negative	Positive for *P. aeruginosa* + *Enterobacter* [2]	-
Clinical sample	Cq < 35	Cq > 35	Positive/Negative	Positive for *P. aeruginosa*	-
Clinical sample	Cq > 35	Cq < 35	Positive/Negative	Positive for *Enterobacter* [2]	-
Clinical sample	Cq > 35	Cq > 35	Negative	Invalid	See 11.2

[1] A positive signal excludes PCR inhibition. However, IPC Cq values should show comparable results among samples. A shift of Cq values can indicate a partial inhibition of PCR.
Exception: Very high pathogen concentrations may result in reduced or no signal from the DNA IPC.

[2] Cross reaction with *Klebsiella* and *Citrobacter* species is possible

In case of invalid data, the analysis must be repeated with the remaining DNA sample or a freshly extracted DNA sample. (see 11. Troubleshooting).

11. Troubleshooting

11.1. No Signal in FAM, VIC and Cy5 channels with controls and samples

- Incorrect programming of the temperature profile on the real-time PCR instrument.
  → Compare the temperature profile with the information in the Instructions for Use.
- Incorrect settings of the detection channels on the real-time PCR instrument.
  → Compare the settings of the detection channels with the information in the Instructions for Use.
- Error in the composition of the PCR reaction
  → Check the pipetting steps using the pipetting scheme in the Instructions for Use and repeat the PCR if necessary.
- No positive control was added.
  → If all clinical samples are also negative, repeat the PCR.

11.2. No signal in Cy5 channel with controls and samples

- Incorrect programming of the temperature profile on the real-time PCR instrument.
  → Compare the temperature profile with the information in the Instructions for Use.
- Incorrect settings of the detection channels on the real-time PCR instrument.
  → Compare the settings of the detection channels with the information in the Instructions for Use.
- Error in the composition of the PCR reaction.
  → Check the pipetting steps using the pipetting scheme in the Instructions for Use and repeat the PCR if necessary.
- The DNA IPC Target was added directly to the master mix, but not freshly diluted 1:100.
  → The PCR reaction is inhibited. Repeat the PCR and add freshly diluted DNA IPC Target.
- The DNA IPC Target was not added during the extraction.
  → Repeat the DNA extraction
- The DNA IPC Target was not pipetted to the lysis buffer but directly to the sample.
  → The DNA IPC Target was degraded. Repeat the DNA extraction.
- The DNA IPC Target was pipetted to the lysis buffer but still no signal in Cy5.
  → DNA extraction has failed or PCR inhibition is present. Check whether a suitable DNA extraction method was used and check the DNA extraction steps.

11.3. Pathogen signal in negative control of extraction (NTC)

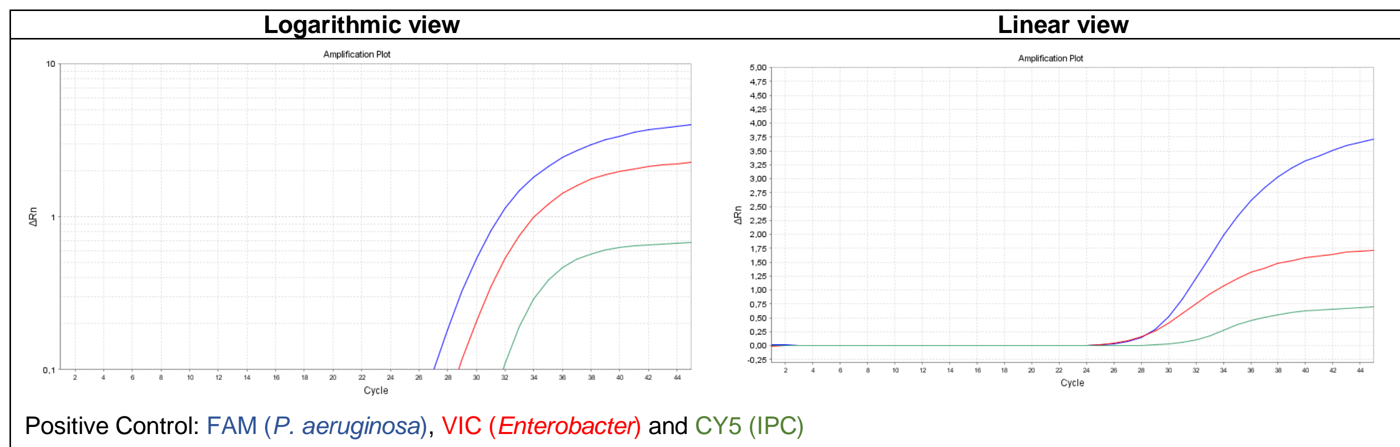
- If there are Cq values in the negative control of extraction below the cut-off specified in the Instructions for Use, a contamination occurred during the preparation of the PCR or the DNA extraction.
  → Repeat the PCR with unused reagents in replicates.
  → Pipette the positive control last.
  → If repeatedly positive: repeat the DNA extraction and PCR with unused reagents.
  → Ensure that work surfaces and equipment are cleaned regularly and that workstations are separated from samples with high bacterial concentrations (e.g. faeces, saliva)

12. Specifications and performance evaluation

12.1. Kit performance

Performance of BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. with an ABI® 7500 instrument (Thermo Fisher Scientific) is shown in Figure 1.

Figure 1 Performance of BactoReal® Kit *P. aeruginosa & Enterobacter* spp.



12.2. Limit of detection, LoD

Method: BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. was tested with a serial dilution of plasmids containing parts of the respective target DNA. The limit of detection (LoD95: number of target copies detected in 95% of cases) was determined with plasmid dilutions around the analytical sensitivity using a non-linear curve fitting with the Graph Pad Prism Software.

Results: The results are shown in Table 3. A cut-off with Cq=35 must be defined for *P. aeruginosa* and *Enterobacter*. The sensitivity of the test (limit of detection, LoD) is reduced accordingly.
Note: Sequence variability in the primer or probe region may lead to false-negative or less sensitive results for some pathogen strains.

Table 3 Summary of LoD and linearity

	LoD95 Without / with cut-off rule	Linearity – Slope	Linearity – correlation coefficient R^2
P. aeruginosa	6 / 50[1]	-3.33 ± 0.02	0.9977
***Enterobacter* spp.**	12 / 50	-3.35 ± 0.03	0.9965

[1]For this organism, a multicopy gene is detected (6 times present in the genome), which increases the actual sensitivity of pathogen detection

12.3. Linearity and dynamic range

Method: Linearity was determined using 10-fold serial dilutions (10^6 – 10^1 target copies/reaction) of the plasmids. The number of determinations (n) per dilution was nine.

Results: Results are shown in Table 3. The test shows linearity over the range of 100 to 1,000,000 target copies/reaction.

12.4. Precision

Precision within a run (intra-assay), between multiple runs (inter-assay) and between two lots (inter-lot) was determined. The mean values of the coefficients of variation (CV%) are summarized in Table 4.

Table 4 Summary of mean values of the coefficients of variation

	Coefficients of variation intra-assay	Coefficients of variation inter-assay	Coefficients of variation inter-lot
P. aeruginosa	0.50	0.46	0.60
***Enterobacter* spp.**	0.37	0.36	0.38

12.5. Analytical specificity

Analytical specificity is ensured by the selection of specific primers and probes. *In silico* validation of primers and probes was performed by examining their sequences for potential homologies to currently published sequences in the NCBI database (BLAST analysis). This database analysis validated the detection of currently known strains and potential cross-reactions.
Existing sequence variability in the target region of some subtypes (strains) may lead to false-negative or less sensitive results.
The results of the BLAST analyses in terms of detection of different species are summarized in Table 5.
Specificity was further determined by testing different bacterial and fungal isolates. No significant cross-reactions were observed (data not shown).

Table 5: Results of BLAST analysis

	Detected species
FAM channel ***Pseudomonas aeruginosa***	*Pseudomonas aeruginosa* Some other *Pseudomonas* strains such as *Delftia acidovorans* (*P. acidovorans*), *P. fluorescens* and *P. otididis.* Not detected are *P. putida* and *Sphingomonas paucimobilis (P. paucimobilis)*
VIC channel ***Enterobacter* spp.**	*E. asburiae, E. bugandensis, E. chengduensis, E. chuandaensis, E. cloacae, E. hormaechei, E. huaxiensis, E. ludwigii, E. oligotrophica, E. mori, E. roggenkampii, E. sichuanensis, E. xiangfangensis,* Lelliottia amnigena *(*formerly *E. amnigenus)* and some *Klebsiella* spp. and *Citrobacter* spp. *E. kobei* is probably detected with lower sensitivity Not detected: *Pluralibacter gergoviae* (*E. gergoviae*) and *E. sakazakii* (*Cronobacter sakazakii*)

12.6. Clinical evaluation

For diagnostic evaluation, 25 blood samples (extracted with SeptiFast LysKit, Roche) and 10 other specimens such as biopsies, CSF and aspirates from patients with suspected bacterial infection were analyzed. The results were compared with the LightCycler® SeptiFast Test (Roche) for blood samples and the SepsiTest™ (Molzym) for the other test materials (see Table 6).

Table 6 Statistical clinical evaluation for blood samples and other samples (biopsies, CSF, aspirates)

	Sensitivity		**Specificity**		**NPV**		**PPV**		**Prevalence**		**Accuracy**	
	Blood	Other	Blood	Other	Blood	Other	Blood	Other	Blood	Other	Blood	Other
P. aeruginosa	90.0%	100.0%	100.0%	85.7%	93.8%	100.0%	100.0%	75.0%	40.0%	30.0%	96.0%	90.0%
Enterobacter	50.00%	100.0%	86.7%	100.0%	72.2%	100.0%	71.4%	100.0%	40.0%	10.0%	72.0%	100.0%

13. Literature

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14. Revision history

Revision	Date	Description

Note:
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Technical support
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