BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp.

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *P. aeruginosa & Enterobacter* spp.			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUS00553	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of *P. aeruginosa, Enterobacter* and of internal DNA positive control (IPC)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction 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 PCR inhibitors)
- DNA positive control for *P. aeruginosa* and *Enterobacter*
- Nuclease-free water

Pathogen 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.

Intended purpose: BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp. is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA of *Pseudomonas aeruginosa* (16S rRNA gene) and *Enterobacter* spp. (rpoB gene).
Proper specimens are DNA extracts isolated from samples of human EDTA blood, CSF aspirates and biopsies.
The test detects the following species: *P. aeruginosa*, *Delftia acidovorans* (*P. acidovorans*), *P. fluorescens, P. otididis, 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* species. *Enterobacter kobei* is detected with lower sensitivity. *Pluralibacter gergoviae* (*E. gergoviae*) and *E. sakazakii* (*Cronobacter sakazakii*) are not detected.
In combination with other diagnostic tests such as culture the test supports a rapid and specific diagnosis for patients suspected of having a bacterial infection. Results must be interpreted in context of the overall picture and other clinical parameters. The diagnosis of sepsis must not be based solely on BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp..
The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.
Probe-specific amplification curves in the fluorescence channel for FAM and VIC detect *P. aeruginosa* and *Enterobacter*, respectively. The Internal DNA 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 IPC (artificial target DNA) is added during sample extraction.

PCR-platforms: This test has been validated with the ABI® 7500 Fast Real-time PCR System (fast cycle parameters are not supported, Thermo Fisher Scientific) and with the cobas z 480 Analyzer (Roche).
It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in the FAM, VIC and Cy5 channel (e.g., QuantStudio™ 5, QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), Mic instrument (bio molecular systems), LightCycler® 480 II (Roche Diagnostics)).

Performance data: The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 50 copies/reaction for both pathogens (corresponds to approx. 12 CFU). A cut-off with Cq=35 must be defined for *P. aeruginosa* and *Enterobacter*. Specificity see intended purpose.
For clinical validation, 25 blood samples and 10 other specimens such as CSF, aspirates and biopsies of patients with suspected bacterial infection were analyzed (see Table 1).

Table 1 Statistical clinical evaluation for blood samples and other specimens

	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%

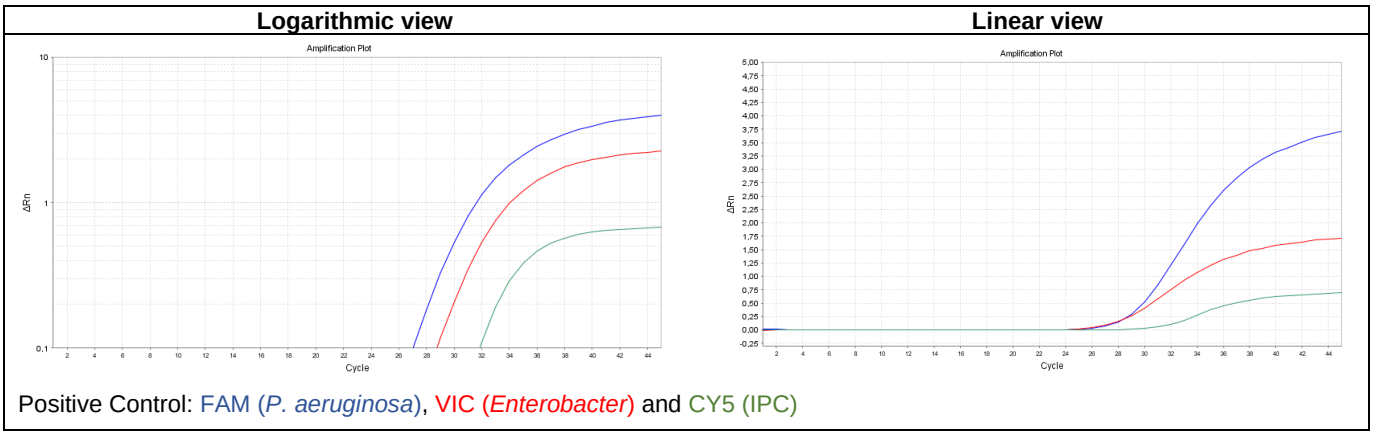

Figure 1 Performance of BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp.

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

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