BactoReal® Kit *Staphylococcus* spp. & *S. aureus*

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Staphylococcus* spp. *& S. aureus*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUS00253	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of *Staphylococcus, S. aureus* 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 *Staphylococcus* and *S. aureus*
- Nuclease-free water

Pathogen information: *Staphylococcus aureus* is a gram-positive bacterium, which can be found on skin and in mucous membranes. This bacterium can cause pneumonia, meningitis, and sepsis. *S. aureus* is a microorganism responsible for nosocomial infections. Some strains are resistant to antibiotics like methicillin and vancomycin and therefore more difficult to treat. *Staphylococcus epidermidis* is a gram-positive bacterium colonizing skin and mucous membranes of humans. It can lead to infections through various implanted medical devices such as joint prostheses, cardiovascular devices, or heart valves.
Staphylococcus saprophyticus can lead to urinary tract infections, with young women being more severely affected.
Other species of staphylococci such as *S. lugdunensis*, *S. haemolyticus*, *S. warneri*, *S. schleiferi*, *S. intermedius* are generally non-pathogenic but can lead to infections in rare cases.

Intended use: BactoReal® Kit *Staphylococcus & S. aureus* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA of *Staphylococcus aureus* and *Staphylococcus* spp.. Proper specimens are DNA extracts isolated from samples of human EDTA blood, aspirates, CSF and biopsies.
The test detects the following species: *Staphylococcus aureus, Streptococcus agnetis, S. argenteus, S. arlettae, S. aureus, S. capitis, S. caprae, S. cohnii, S. delphini, S. epidermidis, S. equorum, S. felis, S. haemolyticus, S. hominis, S. hyicus, S. kloosii, S. lugdunensis, S. lutrae, S. muscae, S. nepalensis, S. pasteuri, S. pettenkoferi, S. piscifermentans, S. pseudintermedius, S. saprophyticus, S. schleiferi, S. sciuri, S. simulans, S. stepanovicii, S. succinus, S. warneri, S. xylosus* and *Macrococcus* spp..
S. carnosus, S. condimenti, *S. chromogenes, S. lutrae* and *S. muscae* will not be detected or detected with low sensitivity.
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 *Staphylococcus & S. aureus*.
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 *Staphylococcus* and *S. aureus,* respectively. The Internal DNA Positive Control (IPC) is detected in the fluorescence channel Cy5 and serves as a control for DNA

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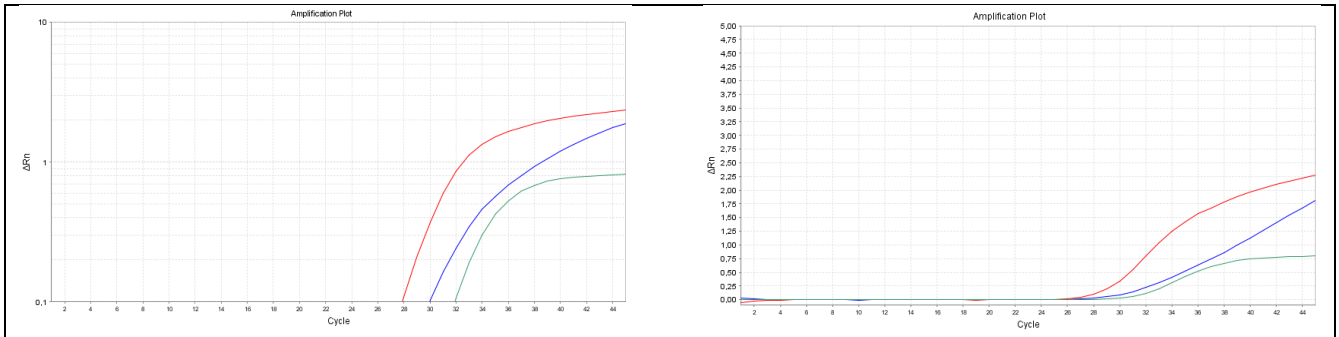
extraction and possible real-time PCR inhibition. The target for the DNA IPC (artificial target DNA) is added during sample extraction.

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)).

Performance data: The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 500 copies/reaction for *Staphylococcus* (corresponds to approx. 83 CFU) and 2 copies/reaction for *S. aureus*. A cut-off with Cq=35 must be defined for *Staphylococcus*. For information on detectable species see intended purpose.
For clinical validation, 27 blood samples and 17 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
Staphylococcus	36.4%	100.0%	100.0%	100.0%	26.3%	100.0%	100.0%	100.0%	81.5%	52.9%	48.1%	100.0%
S. aureus	42.9%	100.0%	100.0%	100.0%	33.3%	100.0%	100.0%	100.0%	77.8%	29.4%	55.6%	100.0%



Positive Control: FAM (*Staphylococcus*), VIC (*S. aureus*) and CY5 (IPC)

Figure 1 Performance of BactoReal® Kit *Staphylococcus & S. aureus*

Further tests for diagnosis of sepsis, which can be combined with *Staphylococcus & S. aureus*:
BactoReal® Kit *E. coli* & *Klebsiella*, order no.: DHUS00153
BactoReal® Kit *S. pneumoniae* & *Streptococcus* spp., order no.: DHUS00353
BactoReal® Kit vanA & *Enterococcus* spp., order no.: DHUS00453
BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp., order no.: DHUS00553
BactoReal® Kit *A. baumannii* & *P. mirabilis*, order no.: DHUS00653
MycoReal® Kit *Candida* & *A. fumigatus*, order no.: DHUF00153

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