BactoReal® Kit vanA & *Enterococcus* spp.

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit vanA & *Enterococcus*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUS00453	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of vanA gene, *Enterococcus* 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 vanA and *Enterococcus*
- Nuclease-free water

Pathogen information: Enterococci are gram-positive, catalase-negative and anaerobic bacteria that play an important role in the digestive system. In immunocompromised individuals, enterococci can lead to urinary tract infections, wound infections and even sepsis. Enterococci are often resistant to vancomycin.
Enterococcus faecalis and *Entercoccus faecium* are the best-known representatives of the *Enterococcus* species. They are used to promote the intestinal flora in probiotic food. However, in people with a weakened immune system, these bacteria can lead to severe infections, including catheter-associated urinary tract infections and sepsis.
Enterococcus casseliflavus, *Enterococcus durans* and *Enterococcus hirae* can rarely lead to infections in humans. *Enterococcus avium* is often found in birds but can lead to infections in humans and may be resistant to vancomycin. *Enterococcus gallinarum* has a low resistance to vancomycin and in rare cases it can lead to infections in humans ranging from meningitis to sepsis.

Intended purpose: BactoReal® Kit vanA & *Enterococcus* spp. is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA of *Enterococcus* spp. (23S rRNA gene) as well as of the vanA resistance gene (vanA) of *Enterococcus.*
The test detects the following species: *E. avium, E. casseliflavus (= E. flavescens), E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus* and *E. saccharolyticus.*
Furthermore, it detects the vanA gene. *E. faecium* is the most common carrier of the vanA gene. However, *E. durans, E. faecalis*, *E. gallinarum, E. saigonensis, E. casseliflavus, E. cecorum*, less commonly *Clostridioides difficile*, *Staphylococcus aureus* and *Staphylococcus haemolyticus* may also carry the vanA gene.
Proper specimens are DNA extracts of samples isolated from samples of human EDTA blood, aspirates, CSF and biopsies.

ingenetix GmbH
Haidingergasse 1, 1030 Vienna, Austria
T +43(0)1 36 198 01
office@ingenetix.com
www.ingenetix.com

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 vanA & *Enterococcus* 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 the vanA gene and *Enterococcus* spp., 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 DNA IPC (artificial target DNA) is added during sample extraction.

PCR-platforms: This test has been validated with the ABI® 7500 Real-time PCR System (Thermo Fisher Scientific, fast cycle parameters are not supported) 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 8 copies/reaction for the vanA gene and 100 copies/reaction for *Enterococcus* (corresponds to approx. 17 CFU). A cut-off with Cq=35 must be defined for *Enterococcus*.
Information on detectable species see intended purpose.
For clinical validation, 20 blood samples and 12 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
Enterococcus	57.1%	100.0%	100.0%	100.0%	50.0%	100.0%	100.0%	100.0%	70.0%	25.0%	70.0%	100.0%

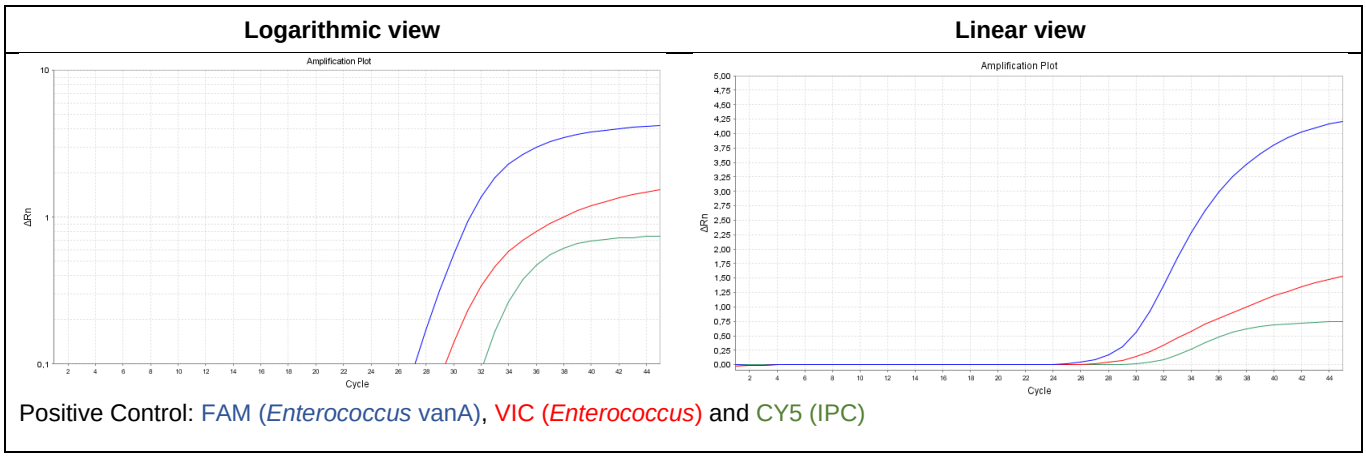

Figure 1 Performance of BactoReal® Kit vanA & *Enterococcus* spp.

Further tests for diagnosis of sepsis, which can be combined with BactoReal® Kit vanA & *Enterococcus* 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 *P. aeruginosa* & *Enterobacter* spp., order no.: DHUS00553
BactoReal® Kit *A. baumannii* & *P. mirabilis*, order no.: DHUS00653
MycoReal® Kit *Candida* & *A. fumigatus*, order no.: DHUF00153

ingenetix GmbH
Haidingergasse 1, 1030 Vienna, Austria
T +43(0)1 36 198 01
office@ingenetix.com
www.ingenetix.com