

The kit is designed to test susceptibility of Gram-positive bacteria to vancomycin on the basis of MIC (minimal inhibitory concentration) determination, i.e. the lowest concentration, which inhibits bacterial growth. The kit contains 36 examinations. The test is based on rehydration of antibiotics in the wells with Mueller Hinton II broth and bacterial suspension. The results are read visually after 24 hours of incubation.

The kit contains:

- 3 plates for examination (36 strips)
- 10 pc of PE bags
- 1 pc of storage bag (for unused strips)

Storage and expiration of the kit:

It is recommended to store the kit at (+2 to +8) °C. The date of expiration is indicated on each package. Leave plate at room temperature for at least 30 minutes before you open it to avoid water condensation.

After the aluminium package is opened and covering foil is removed, don't leave opened plates unprotected!!! Exposition to air humidity leads to antibiotic activity failure!!!

Material required to perform a test, not included in the kit:

- Sterile physiological solution
- Muller Hinton broth II cation-adjusted (e.g. Suspension medium MIC, Cat. No. MLT00070) – for staphylococci
- Suspension medium MIC G+ (Cat. No. MLT00071) or MHB supplemented with lysed horse blood and β-NAD (MH-F broth), more on www.eucast.org – for enterococci and streptococci
- Sterile tubes
- Sterile basins 60 ml for pipetting Cat. No. 50004457
- Frames for strips Cat. No. 50004477
- A stepper or multichannel pipette for dosage of 100 µl
- A pipette for dosage of 20–100 µl
- Instrument to measure optical density of bacterial inoculum (e.g. DENSILAMETER II Cat. No. INS00062)
- Incubator 35±2 °C
- Regular microbiological laboratory equipment (loops, marker, burner, etc.)

Caution: The kit is for professional use only! Respect the rules for work with infectious material!

Instructions for Use

Preparation of bacterial suspension (recommended procedure):

- 1) Prepare a tube with physiological solution.
- 2) Remove few colonies from 18–24 hour culture on blood agar and prepare a bacterial suspension of density of 0.5 on McFarland scale in physiological solution.
- 3) Inoculate 120 µl of staphylococci suspension into a tube with 13 ml of Suspension medium MIC. When testing enterococci or streptococci, transfer 60 µl of bacterial suspension into a tube with 13 ml of Suspension medium MIC G+. Homogenise suspensions well.

Inoculation:

Remove a plate from aluminium bag and remove aluminium cover from a strip, e.g. use a scalpel or a ruler (just before inoculation). **Don't leave opened plates unprotected!!! Exposition to open air leads to antibiotic activity failure!!!** Insert any unused strips into a ZIP log storage bag. Transfer a dessicant sachet from the original package into a storage bag as well and close it for later use. Store it at 2–8 °C for 4 weeks maximum.

Record number of the examined strain on the strip and insert it into a free frame. Inoculate 100 µl of suspension medium with bacterial inoculum into each well of the strip. Note: Process the strips within 60 minutes after removing it from aluminium bag.

Incubation:

Insert the inoculated strip in a frame into a PE bag. Fold the open end of the bag under the plate to prevent evaporation during the incubation. Incubate the plate at 35±2 °C for 24 hours.

Evaluation:

Remove the plate from the PE bag. To read the growth in the microwells, choose a way which is the most convenient for you:

- 1) Read against a grey background or against plate layout in the instructions.
- 2) Read against natural or artificial dispersed light.

Please read with attention:

You must see a growth in the control well (placed in row H)! If the growth is not present, the test MUST NOT be evaluated! The MIC is the lowest concentration of antibiotic in a well where no visible growth of the organism is observed. Beware to differentiate grains of growth from media bubbles. Record the results.

Tab. 1: Plate layout: vancomycin dilution series (in mg/l)

A	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16	VAN 16
B	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8	VAN 8
C	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4	VAN 4
D	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2	VAN 2
E	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1	VAN 1
F	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5	VAN 0.5
G	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25	VAN 0.25
H	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control	Growth control
	Vancomycin 1	Vancomycin 2	Vancomycin 3	Vancomycin 4	Vancomycin 5	Vancomycin 6	Vancomycin 7	Vancomycin 8	Vancomycin 9	Vancomycin 10	Vancomycin 11	Vancomycin 12

Tab. 2: Clinical MIC breakpoints (mg/l) for vancomycin

	EUCAST			CLSI		
	susceptible	intermediate	resistant	susceptible	intermediate	resistant
<i>Staphylococcus aureus</i>	≤ 2		≥ 4	≤ 2	4-8	≥ 16
<i>Coagulase negative staphylococci</i>	≤ 4		≥ 8	≤ 4	8-16	≥ 32
<i>Enterococcus spp.</i>	≤ 4		≥ 8	≤ 4	8-16	≥ 32
<i>Streptococcus gr. A, B, C and G (β-hemolytic CLSI), S. pneumoniae</i>	≤ 2		≥ 4	≤ 1		-

ATU (Areas of Technical Uncertainty) - before interpretation of results:

- Repeat the test
- Use an alternative test
- Downgrade the susceptibility category
- Include the uncertainty as part of the report

More on www.eucast.org

Interpretation:

The tested strain is categorized as susceptible-intermediate-resistant to vancomycin on the basis of MIC determination according to EUCAST interpretation tables (1) or according to CLSI document M100 (2).

VAN resistant strains should be referred to a reference laboratory. In case of enterococci, it is important to ensure that the isolate is not *E. gallinarum* or *E. casseliflavus* which have intrinsic resistance to vancomycin. To detect resistance to vancomycin follow EUCAST guidelines (3) or CLSI dokument M100 (2). Other interpretive criteria must be used depending on national and laboratory standards, e.g. EUCAST Expert rules (4) or CLSI dokument M100 (2) and M07 (5). It is necessary to take following parameters into consideration when interpreting results: species identification, sample origin, patient case history, or results of additional tests.

Quality control:

We recommend following control strains for internal testing of functionality of the antibiotics in the laboratory. Follow EUCAST or CLSI standards when evaluating results.

CCM 4223 (ATCC 29213) <i>Staphylococcus aureus</i> MIC (mg/l)	CCM 4224 (ATCC 29212) <i>Enterococcus faecalis</i> MIC (mg/l)
VAN 0.5-2	VAN 1-4

ATCC – American Type Culture Collection

CCM – Czech Collection of Microorganisms, Masarykova univerzita, Faculty of Science, Kamenice 5, budova A25, 625 00 Brno, tel. 549 491 430, fax 549 498 289, <http://www.sci.muni.cz/ccm>, e-mail: ccm@sci.muni.cz

Health protection:

Components of the kit are not classified as dangerous.

Disposal of the used material:

Insert the used plate into the vessel intended for the infectious material and autoclave or destroy it by incineration.

Literature:

- 1) The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MIC and zone diameters, <http://www.eucast.org>
- 2) CLSI: Performance Standards for Antimicrobial Susceptibility Testing; CLSI dokument M100. Wayne, PA: Clinical and Laboratory Standards Institute.
- 3) EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, <http://www.eucast.org>
- 4) EUCAST expert rules in antimicrobial susceptibility testing, <http://www.eucast.org>
- 5) CLSI: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; CLSI dokument M07. Wayne, PA: Clinical and Laboratory Standards Institute.

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USED SYMBOLS



Catalogue number



In vitro diagnostics



Manufacturer



See instruction for use



Lot number



Storage temperature



Expiry date

