CE IVD

MIC G+

EN

Cat. N.: MLT00043

For microbiology

The kit is designed to test antimicrobial susceptibility of enterococci and streptococci (gr. A, B, C, G and *S. pneumoniae*) on the basis of MIC determination (minimal inhibitory concentration), i.e. the lowest concentration, which inhibits bacterial growth. The kit contains 10 examinations (plates). The test is based on the rehydration of antibiotics in the wells with suspension medium and addition of bacterial suspension. The results are read visually or by reader after 16–20 hours of incubation.

The kit contains:

- 10 plates for examination
- A lid (non-sterile)
- 10 pc of PE bags

Storage and expiration of the kit:

It is recommended to store the kit at (+2 to +25)°C. The date of expiration is indicated on each package. Leave plate at room temperature at least 30 minutes before you open it to avoid water condensation. After the aluminium package is opened, 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:

- Suspension medium MIC G+ (cat. N. MLT00071) or MHB supplemented with lysed horse blood and β-NAD (MH-F broth), more on www.eucast.org
- Sterile physiological solution (unbuffered)
- Ethanol
- Sterile tubes
- Inoculator ErbaDip (Cat. N. 50004456)
- Sterile Petri dishes
- Sterile basins 60 ml (Cat. N. 50004457)
- A stepper or multichannel pippete for dosage of 100 µl
- A pippete for dosage of 60-100 µl
- Densitometer (e.g. DENSILAMETER II, Cat. N. 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 and inoculation (recommended procedure):

A) Inoculation with inoculator

1) Remove a plate from aluminium bag and remove aluminium cover. Mark the frame with a type of kit (G+) to avoid mistake in reading results after incubation. Record number of examined strain on the plate. Fill 100 µl of suspension medium MIC G+ into each well.
2) Prepare a tube with 12 ml of physiological solution. When inoculating with an inoculator, add 100 µl of suspension medium MIC G+ to decrease surface tension.
3) Remove few colonies from 18–24 hour culture on blood agar and prepare a bacterial suspension of density of 0.5 on McFarland scale for enterococci and 1.0 on McFarland scale for streptococci in physiological solution.
4) Pour the bacterial suspension into a sterile Petri dish.
5) Use sterile inoculator to inoculate the plate: dip inoculator into Petri dish with ethanol and flame it. Dip the cooled inoculator into a Petri dish with prepared bacterial suspension. A thin film of bacterial suspension is adhered to metal spikes of inoculator. Transfer inoculum to the first half of the plate by dipping into wells and carefull mixing. Make a new dip into the Petri dish with prepared bacterial inoculum and inoculate the second half of the plate.

B) Inoculation with pippete

1) Prepare a tube with 2 ml of 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) Place 60 µl of bacterial suspension into a tube with 13 ml of suspension medium MIC, homogenise well.
4) Remove a plate from aluminium bag and remove aluminium cover from the plate. Mark the frame with a type of kit (G+) to avoid mistake in reading results after incubation. Record number of the examined strain on the plate.
5) Inoculate each well of the plate with 100 µl of bacterial suspension prepared in suspension medium MIC.

Note: Process a plate within 60 minutes after removing it from aluminium bag.

Incubation:

Insert the inoculated plate 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 16–20 hours. The plate with enterococci is incubated for 24 hours before vancomycin is read.

Evaluation:

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

1) Read against a grey background or against plate layout in instructions.
2) Read against natural or artifical dispersed light.
3) Usage of magnifying glass is not recommended.
4) Evaluate test using reader and identification software.

Please read with attention:

You must see a growth in the control well (K)! 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. Exeption: With Trimethoprim/sulfamethoxazol, a well with ≥ 80% growth inhibition compered to the growth control is considered as MIC. Beware to differentiate grains of growth from media bubbles. Record the results.

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

	1 PEN	2 AMP	3 ERY	4 CLI	5 LIZ	6 CMP	7 TET	8 T/S	9 GEN	10 VAN	11 TEC	12 NFT
A	8	16	8	16	16	32	32	4/76	128	16	16	128
B	4	8	4	8	8	16	16	2/38	16	8	8	64
C	2	4	2	4	4	8	8	1/19	8	4	4	32
D	1	2	1	2	2	4	4	0.5/9.5	4	2	2	16
E	0.5	1	0.5	1	1	2	2	0.25/4.75	2	1	1	8
F	0.25	0.5	0.25	0.5	0.5	1	1	0.12/2.38	1	0.5	0.5	4
G	0.12	0.25	0.12	0.25	0.25	0.5	0.5	0.06/1.19	0.5	0.25	0.25	2
H	0.06	0.12	0.06	0.12	0.12	0.25	0.25	0.03/0.6	0.25	0.12	0.12	K

Tab 2: Clinical MIC breakpoints (in mg/l) for enterococci and streptococci according to EUCAST (1)

		enterococci			streptococci		
Antibiotics	**Abbr.**	**Sensitive S**	**Intermediate I**	**Resistant R**	**Sensitive S**	**Intermediate I**	**Resistent R**
Penicillin	PEN	-	-	-	≤0.25 gr. A,B,C,G		≥0.5 gr. A,B,C,G
					≤0.06 *S. pneumoniae*	0.12-2 *S. pneumoniae*	≥4 *S. pneumoniae*
					≤0.06 *S. pneumoniae* (meningitis)		≥0.12 *S. pneumoniae* (meningitis)

Ampicillin	AMP	≤4	8	≥16	≤0.5 *S. pneumoniae*	*1-2* *S. pneumoniae*	≥4 *S. pneumoniae*
Erythromycin	ERY	-	-	-	≤0.25	0.5	≥1
Clindamycin	CLI	-	-	-	≤0.5		≥1
Linezolid	LIZ	≤4		≥8	≤2		≥4
Chloramphenicol	CMP	-	-	-	≤8		≥16
Tetracycline	TET	-	-	-	≤1	2	≥4
*Trimethoprim / sulfamethoxazole	T/S				≤1/19	2/38	≥4/76
Gentamicin (test for high-level aminoglycoside resistance)*	GEN				-	-	-
Vancomycin	VAN	≤4		≥8	≤2		≥4
Teicoplanin	TEC	≤2		≥4	≤2		≥4
Nitrofurantoin	NFT	≤64 *E. faecalis*		≥128 *E. faecalis*	≤64 *S. agalactiae*		≥128 *S. agalactiae*

*E. faecalis and E. faecium ECCOF = 1
*MIC ≤128 = negative test
MIC ≥256 = positive test
More on www.eucast.org

Tab 3: Clinical MIC breakpoints (in mg/l) for enterococci and streptococci according to CLSI (2)

		enterococci			streptococci		
Antibiotics	**Abbr.**	**Sensitive S**	**Intermediate I**	**Resistant R**	**Sensitive S**	**Intermediate I**	**Resistent R**
Penicillin	PEN	≤8		≥16	≤0.12 gr. A,B,C,G		- gr. A,B,C,G
					≤2 S. pneumoniae	4 S. pneumoniae	≥8 S. pneumoniae
					≤0.06 *S. pneumoniae* (meningitis)	- S. pneumoniae (meningitis)	≥0,12 S. pneumoniae (meningitis)
Ampicillin	AMP	≤8		≥16	≤0.25 gr. A,B,C,G		
Erythromycin	ERY	≤0.5	1-4	≥8	≤0.25	0.5	≥1
Clindamycin	CLI				≤0.25	0.5	≥1
Linezolid	LIZ	≤2	4	≥8	≤2		-
Chloramphenicol	CMP	≤8	16	≥32	≤4 gr. A,B,C,G	8 gr. A,B,C,G	≥16 gr. A,B,C,G
					≤4 S. pneumoniae		≤8 *S. pneumoniae*
Tetracycline	TET	≤4	8	≥16	≤2 gr. A,B,C,G	4 gr. A,B,C,G	≥8 gr. A,B,C,G
					≤1 S. pneumoniae	2 S. pneumoniae	≥4 S. pneumoniae
Trimethoprim / sulfamethoxazole	T/S				≤0.5/9.5 S. pneumoniae	1/19-2/38 S. pneumoniae	≥4/76 S. pneumoniae
Gentamicin	GEN						
Vancomycin	VAN	≤4	8-16	≥32	≤1		-
Teicoplanin	TEC	≤8	16	≥32			
Nitrofurantoin	NFT	≤32	64	≥128			

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

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Interpretation: The tested strain is categorised as sensitive-intermediate-resistant to a particular antibiotic on the basis of MIC determination. This cathegorisation is based on EUCAST (1) or according to CLSI document M100 (2).

Enterococci: Ampicillin: Results can be also used for amoxicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam.

Streptococci: Penicillin: Susceptibility of streptococci gr. A, B, C, G to beta-lactams (including ampicillin) is inferred from the penicillin susceptibility. Strains resistant to penicillin are rare or not described – such strains should be reffered to a reference laboratory. Beta hemolytic streptococci do not produce beta-lactamase, therefore their combination with inhibitors does not add any benefitt. Breakpoints for penicillins other than benzylpenicillin relate only to non-meningitis isolates.

Erythromycin: Results can be interpreted for pro azithromycin, clarithromycin a roxithromycin.

Clindamycin: If resistant to erythromycin and sensitive to clindamycin (or clindamycin intermediary) determine induction of resistance to clindamycin by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as susceptible and add a comment according to recommendation of EUCAST (1) or report as resistant and add a comment according to recommendation of CLSI (2).

Teicoplanin: If resistant confirm by another test and refer to a reference laboratory. Resistant strains are rare.

Other interpretative criteria have to be used depending on national and laboratory standards. EUCAST Expert rules 3 or CLSI documents M100 (2) and M07 (4). It is necessary to take into consideration following parameters when interpreting results: species identification, sample origin, patient case history, or resuls of additional tests.

Quality control: We recommend all following control strain for internal testing of functionality of the antibiotics in the laboratory. Follow EUCAST or CLSI standards when evaluating results. Fresh strains must be used for quality control.

CCM 4224 *Enterococcus faecalis* (ATCC 29212) MIC (mg/l)											
PEN 1-4	AMP 0,5-2	ERY 1-4	CLI 4-16	LIZ 1-4	CMP 4-16	TET 8-32	T/S ≤0.5/9.5	GEN 4-16	VAN 1-4	TEC 0.25-1	NFT 4-16

CCM 4501 *Streptococcus pneumoniae* (ATCC 49619) MIC (mg/l)											
PEN 0.25-1	AMP 0.06-0.25	ERY 0.03-0.12	CLI 0.03-0.12	LIZ 0.25-2	CMP 2-8	TET 0.06-0.5	T/S 0.12/2.4-1/19	GEN -	VAN 0.12-0.5	TEC -	NFT 4-16

ATCC – American Type Culture Collection
CCM – Czech Collection of Microorganisms, Masaryk University, Faculty of Science, Kamenice 5, building A25, 625 00 Brno, CZ
Tel. +420 549 491 430, Fax +420 549 498 289, http://www.sci.muni.cz/ccm, e-mail: ccm@sci.muni.cz

Health protection: Components of the kit do not contain any dangerous substances.

Disposal of the used material: Insert the used plate into the vessel intended for the infectious material and autoclave or destroy it by incineration. Put paper packaging waste to recycling.

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 expert rules in antimicrobial suceptibility testing; http://www.eucast.org
(4) CLSI: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. CLSI dokument M07. Wayne, PA: Clinical and Laboratory Standards Institute.

USED SYMBOLS

Date of revision: 5. 3. 2020

REF Catalogue number | IVD In vitro diagnostics | Manufacturer | See instruction for use

LOT Lot number | Storage temperature | Expiry date

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