CE IVD

MIC NEFERM

EN

Cat. N.: MLT00046

For microbiology

The kit is designed to test antimicrobial susceptibility of Gram-negative non-fermentative bacteria on the basis of MIC (minimal inhibitory concentration) determination, 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 Muller Hinton II broth 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 for 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:

- Sterile physiological solution (unbuffered)
- Muller Hinton broth II cationts adjusted (e.g. suspension medium MIC Cat. N. MLT00070)
- 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 (NEFERM) to avoid mistake in reading results after incubation. Record number of examined strain on the plate. Fill 100 µl of suspension medium MIC into each well.
2) Prepare a tube with 12 ml of physiological solution. Add 100 µl of suspension medium MIC 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 McF scale in physiological solution. It is necessary to give extra attention to achieving a smooth suspension of 0.5 McFarland when preparing inoculum of *Pseudomonas spp.* The growth method can be used alternativelly: 3-5 colonies are transfered into a tube with suitable broth medium and incubated until the turbidity of 0.5 Mc Farland is achieved or exceeded. The turbidity of the broth culture is adjusted with sterile saline or broth to 0.5 Mc Farland standard.
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. It is necessary to give extra attention to achieving a smooth suspension of 0.5 McFarland when preparing inoculum of Pseudomonas spp. The growth method can be used alternativelly: 3-5 colonies are transfered into a tube with suitable broth medium and incubated until the turbidity of 0.5 Mc Farland is achieved or exceeded. The turbidity of the broth culture is adjusted with sterile saline or broth to 0.5 Mc Farland standard.
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 (NEFERM) 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. If MIC can not be evaluated in slowly growing strains (e.g. *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *Pseudomonas aeruginosa* from cystic fibrosis) extend the incubation for another 20 hours at room temperature.

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 Trimehtoprim/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 AMS	2 PIP	3 PIT	4 CAZ	5 AZT	6 MER	7 GEN	8 AMK	9 COL	10 CIP	11 TGC	12 T/S
A	128/64	128	128/4	16	16	16	32	64	16	8	8	4/76
B	64/32	64	64/4	8	8	8	16	32	8	4	4	2/38
C	32/16	32	32/4	4	4	4	8	16	4	2	2	1/19
D	16/8	16	16/4	2	2	2	4	8	2	1	1	0.5/9.5
E	8/4	8	8/4	1	1	1	2	4	1	0.5	0.5	0.25/4.75
F	4/2	4	4/4	0.5	0.5	0.5	1	2	0.5	0.25	0.25	0.12/2.38
G	2/1	2	2/4	0.25	0.25	0.25	0.5	1	0.25	0.12	0.12	0.06/1.19
H	1/0.5	1	1/4	0.12	0.12	0.12	0.25	0.5	0.12	0.06	0.06	K

Tab. 2: Clinical MIC Breakpoints (in mg/l) for Gram-negative non-fermentative bacteria according to EUCAST (1)

Antibiotics	Abbr.	*Pseudomonas sp.*			*Stenotrophomonas maltophilia*			*Acinetobacter spp.*		
		Sensitive S	Inter-mediate I	Resistent R	Sensitive S	Inter-mediate I	Resistent R	Sensitive S	Inter-mediate I	Resistent R
Ampicillin / sulbactam	AMS	-	-	-				IE		IE
Piperacillin	PIP	≤16		≥32				IE		IE
Piperacillin / tazobactam	PIT	≤16/4		≥32/4				IE		IE
Ceftazidime	CAZ	≤8		≥16				-	-	-
Aztreonam	AZT	≤16	-	≥32				-	-	-
Meropenem	MER	≤2	4-8	≥16				≤2	4-8	≥16
Gentamicin	GEN	≤4		≥8				≤4		≥8
Amikacin	AMK	≤8	16	≥32				≤8	16	≥32
Colistin	COL	≤2		≥4*				≤2		≥4
Ciprofloxacin	CIP	≤0.5		≥1				≤0,06	0,12-1	≥2
Tigecycline	TGC	-	-	-				IE		IE
Trimethoprim/sulfamethoxazole	T/S	-	-	-	≤4/76		≥8/152	≤2/38	4/76	≥8/152

*applies to MIC = 4

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

Tab. 3: Clinical MIC Breakpoints (in mg/l) for Gram-negative non-fermentative bacteria according to CLSI (2)

Antibiotics	Abbr.	*Pseudomonas aeruginosa*			*Stenotrophomonas maltophilia Burkholderia cepacia*			*Acinetobacter spp.*		
		Sensitive S	Inter-mediate I	Resistent R	Sensitive S	Inter-mediate I	Resistent R	Sensitive S	Inter-mediate I	Resistent R
Ampicillin / sulbactam	AMS							≤8/4	16/8	≥32/16
Piperacillin	PIP	≤16	32-64	≥128				≤16	32-64	≥128
Piperacillin / tazobactam	PIT	≤16/4	32/4-64/4	≥128/4				≤16/4	32/4-64/4	≥128/4
Ceftazidime	CAZ	≤8	16	≥32	≤8	16	≥32	≤8	16	≥32
Aztreonam	AZT	≤8	16	≥32						
Meropenem	MER	≤2	4	≥8	≤4 only B. cepacia	8 only B. cepacia	≥16 only B. cepacia	≤2	4	≥8
Gentamicin	GEN	≤4	8	16				≤4	8	≥16
Amikacin	AMK	≤16	32	≥64				≤16	32	≥64
Colistin	COL	≤2		≥4				≤2		≥4
Ciprofloxacin	CIP	≤0,5	1	≥2				≤1	2	≥4
Tigecycline	TGC									
Trimethoprim/sulfamethoxazole	T/S				≤2/38		≥4/76	≤2/38		≥4/76

Interpretation:

The tested strain is categorised as sensitive-intermediate-resistant to a particular antibiotic on the basis of MIC determination. This categorisation is based on EUCAST: Breakpoint (1) or according to CLSI document M100 (2). „IE" indicates that there is insufficient evidence that the species in question is a good target for therapy with the drug. An MIC with a comment but without an accompanying S, I or R categorisation may be reported.

Other interpretative criteria have to be used depending on national and laboratory standards, e.g. 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. We recommend to evaluate MIC only for antibiotics recommended by EUCAST or CLSI for *S. maltophilia* and *B. cepacia*.

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 3955 *Pseudomonas aeruginosa* (ATCC 27853) MIC (mg/l)											
AMS	PIP	PIT	CAZ	AZT	MER	GEN	AMK	COL	CIP	TGC	T/S
-	1-8	1/4-8/4	1-4	2-8	0.25-1	0.5-2	1-4	0.5-4	0.25-1	-	-

CCM 3954 *Escherichia coli* (ATCC 25922) MIC (mg/l)											
AMS	PIP	PIT	CAZ	AZT	MER	GEN	AMK	COL	CIP	TGC	T/S
2/1-8/4	1-4	1/4-4/4	0.06-0.5	0.06-0.25	0.008-0.06	0.25-1	0.5-4	0.25-2	0.004-0.015	0.03-0.25	≤0.5/9.5

CCM 4225 *Escherichia coli* (ATCC 35218) MIC (mg/l)											
AMS	PIP	PIT									
8/4-32/16	>64	0.5/4-2/4									

NCTC 13846 *Escherichia coli* (mcr-1 positive) MIC (mg/l)											
-	-	-	-	-	-	-	COL 4* (exceptionally 2-8)	-	-	-	-

*The value is valid for ≥ 90% isolates and should only on occasion be 2 or 8 mg/L (source: EUCAST)

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 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. 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; 2018.
(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.

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USED SYMBOLS

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

LOT Lot number | Storage temperature | Expiry date

10020297

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