



Cat. N.: MLT00048

For microbiology

The kit is designed to test antimicrobial susceptibility of bacteria isolated from urine and urinary tract (mainly *Enterobacteriaceae* family) 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!!! Exposure 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 cations adjusted (e.g. suspension medium MIC Cat. N. MLT00070)
- Ethanol
- 1) Sterile tubes
- Inoculator ErbaDip Cat. N. 50004456
- Sterile Petri dishes
- Sterile basins
- A stepper or multichannel pipette for dosage of 100 µl
- A pipette 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 (URINE) 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 McFarland scale 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 careful mixing. Make a new dip into the Petri dish with prepared bacterial inoculum and inoculate the second half of the plate.

**B) Inoculation with pipette**

- 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 (URINE) 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.

**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 instructions.
- 2) Read against natural or artificial 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. Exception: With Trimethoprim/sulfamethoxazol, a well with ≥ 80% growth inhibition compared 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	2	3	4	5	6	7	8	9	10	11	12
	AMP	AMS	CFZ	CXM	MER	GEN	AMK	T/S	NOR	CIP	TGC	NFT
A	128	128/64	16	64	16	32	64	4/76	8	8	8	128
B	64	64/32	8	32	8	16	32	2/38	4	4	4	64
C	32	32/16	4	16	4	8	16	1/19	2	2	2	32
D	16	16/8	2	8	2	4	8	0.5/9.5	1	1	1	16
E	8	8/4	1	4	1	2	4	0.25/4.75	0.5	0.5	0.5	8
F	4	4/2	0.5	2	0.5	1	2	0.12/2.38	0.25	0.25	0.25	4
G	2	2/1	0.25	1	0.25	0.5	1	0.06/1.19	0.12	0.12	0.12	2
H	1	1/0.5	0.12	0.5	0.12	0.25	0.5	0.03/0.6	0.06	0.06	0.06	K

Tab 2: Clinical MIC breakpoints (in mg/l) for *Enterobacteriaceae*

Antibiotics	Abbr.	EUCAST			CLSI		
		Sensitive S	Intermediate I	Resistant R	Sensitive S	Intermediate I	Resistant R
Ampicillin	AMP	≤8		≥16	≤8	16	≥32
Ampicillin / sulbactam	AMS	≤8/4		≥16/4	≤8/4	16/8	≥32/16
Cefazolin (infections originating from the urinary tract) <i>E. coli</i> , <i>Klebsiella</i> spp. (except <i>K. aerogenes</i> )	CFZ	≤0.001	0.002-4	≥8	≤2	4	≥8
Cefuroxime parenteral <i>EE. coli</i> , <i>Klebsiella</i> spp. (except <i>K. aerogenes</i> ), <i>Raoultella</i> spp. and <i>P. mirabilis</i>	CXM	≤0.001	0.002-8	≥16	≤8	16	≥32
Cefuroxime oral (uncomplicated UTI only) <i>E. coli</i> , <i>Klebsiella</i> spp. (except <i>K. aerogenes</i> ), <i>Raoultella</i> spp. and <i>P. mirabilis</i>		≤8		≥16			
Meropenem	MER	≤2	4-8	≥16	≤1	2	≥4
Gentamicin (systemic infections)	GEN	(≤2)		(≥4)	≤4	8	≥16
Gentamicin (infections originating from the urinary tract)		≤2	≥4				
Amikacin (systemic infections)	AMK	(≤8)		(≥16)	≤16	32	≥64
Amikacin (infections originating from the urinary tract)		≤8	≥16				
Trimethoprim / sulfamethoxazole	T/S	≤2/38	4/76	≥8/152	≤2/38		≥4/76
Norfloxacin (uncomplicated UTI only)	NOR	≤0.5		≥1	≤4	8	≥16
Ciprofloxacin	CIP	≤0.25	0,5	≥1	≤1	2	≥4
Ciprofloxacin, <i>Salmonella</i> spp.		≤0.06		≥0.12			
Tigecycline	TGC	≤1	2	≥4			
Nitrofurantoin	NFT	≤64		≥128	≤32	64	≥128

**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 tables (1) or according to CLSI document M100 (2).

1) Resistance to CXM, CFZ indicates possibility of ESBL production. MER is recommended to screen for Carbapenemases production. Confirmation tests are recommended according to EUCAST guidelines (3) or CLSI document M100 (2).

2) It is necessary to correct susceptibility of some strains to beta-lactam antibiotics (mainly *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., *Serratia* spp.) to resistant according to their intrinsic resistance. In this case beta-lactamase is not produced in sufficient amount *in vitro*.

3) Aminoglycosides (EUCAST) - for systemic infections, aminoglycosides must be used in combination with other active therapy. In this circumstance, the breakpoint in brackets can be used to distinguish between organisms with and without acquired resistance mechanisms. For isolates without resistance mechanisms, include a comment in the report: "Aminoglycosides are often given in combination with other agents, either to support the activity of the aminoglycoside or to broaden the spectrum of therapy. In systemic infections, the aminoglycoside must be supported by other active therapy."

Other interpretative criteria have to be used depending on national and laboratory standards, e.g. EUCAST Expert rules (4) or CLSI documents M100 (2) and M07 (5). It is necessary to take into consideration following parameters when interpreting results: species identification, sample origin, patient case history, or results of additional tests.

**Quality control:**

We recommended all following control strains 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 3954 <i>Escherichia coli</i> (ATCC 25922)											
MIC (mg/l)											
AMP	AMS	CFZ	CXM	MER	GEN	AMK	T/S	NOR	CIP	TGC	NFT
2-8	2/1-8/4	1-4	2-8	0.008-0.06	0.25-1	0.5-4	≤0.5/9.5	0.03-0.12	0.004-0.015	0.03-0.25	4-16

CCM 3955 <i>Pseudomonas aeruginosa</i> (ATCC 27853)											
MIC (mg/l)											
AMP	AMS	CFZ	CXM	MER	GEN	AMK	T/S	NOR	CIP	TGC	NFT
-	-	-	-	0.125-1	0.5-2	1-4	8/152-32/608	1-4	0.125-1	-	-

CCM 4225 <i>Escherichia coli</i> (ATCC 35218)											
MIC (mg/l)											
AMP	AMS										
> 32	8/4-32/16										

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](mailto: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.

(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

10020299