

DISKS FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

50 DISKS CARTRIDGE

Study of susceptibility to antimicrobial agents.



2020/05

1. INTENDED USE

Antibiotic disks are used to perform a semi-quantitative antimicrobial susceptibility testing using disk diffusion method.

2. SUMMARY AND EXPLANATION OF THE TEST

These disks are used to semi quantitatively evaluate the *in vitro* susceptibility to antimicrobial agents of rapidly growing bacteria and several difficult species by an agar diffusion method.

This method is based on a standardized procedure published by the WHO^(1,2) and adopted as consensual standard by the CLSI⁽⁴⁾ CA-SFM/EUCAST⁽⁶⁾ and EUCAST⁽⁹⁾ (it is periodically revised).

Consult the most recent CLSI^(4,5), CA-SFM/EUCAST⁽⁶⁾, EUCAST^(7,8) documents for guidelines concerning antibiotic susceptibility testing and interpretation of the results.

3. PRINCIPLE OF THE PROCEDURE

Paper disks impregnated with a defined concentration of antimicrobial agent are deposited on the surface of an appropriate medium^(A) previously inoculated with a calibrated inoculum^(A) of pure and fresh culture of the bacterial strain to be tested. After incubation^(A), the Petri dishes are examined and the zones of inhibition around the disks are measured and compared to critical values^(A) for the various antimicrobial agents tested, in order to determine the clinical category of susceptibility (resistant, intermediate, susceptible...). The diameter of the zone of inhibition is proportional to the susceptibility of the bacterial strain tested.

^(A)According to current guidelines CLSI⁽⁴⁾, EUCAST⁽⁹⁾.

4. REAGENTS

4.1 Description

Bio-Rad disks are 6.5 mm disks made from superior quality absorbent paper and impregnated with precise concentrations of antimicrobial agents. The disks are clearly identified by a code; comprising 3 letters, printed on each side of the disk (see Table 1). Bio-Rad disks are supplied in cartridges of 50 disks packaged in watertight containers containing a desiccant.

Table 1: Bio-Rad disks for antibiotic susceptibility testing

	DISK CONTENT	SYMBOL	PACK SIZE	Product code
Amikacin	30 µg	AKN30	4 x 50 Disks	66148
Amoxicillin	20 µg	AMO20	4 x 50 Disks	68042
Amoxicillin + Clavulanic Acid	2/1 µg 20/10 µg	AUG3 AMC30	4 x 50 Disks 4 x 50 Disks	66680 66178
Ampicillin	2 µg 10 µg	API2 AMP10	4 x 50 Disks 4 x 50 Disks	67288 66128
Ampicillin + Sulbactam	10/10 µg	SAM20	4 x 50 Disks	67018
Azithromycin	15 µg	AZM15	4 x 50 Disks	67008
Aztreonam	30 µg	ATM30	4 x 50 Disks	66928
Bacitracin	10 IU (130 µg)	BCT130	4 x 50 Disks	66158
Benzylpenicillin	1 IU	PNG1	4 x 50 Disks	67788
Carbenicillin	100 µg	CRB100	4 x 50 Disks	66198
Cefaclor	30 µg	CEC30	4 x 50 Disks	67498
Cefalexin	30 µg	CXN30	4 x 50 Disks	66208
Cefamandole	30 µg	FAM30	4 x 50 Disks	66238
Cefazolin	30 µg	CZN30	4 x 50 Disks	66258
Cefepime	30 µg	FEP30	4 x 50 Disks	66098
Cefixime	5 µg	FIX5	4 x 50 Disks	67588
Cefoperazone	75 µg 30 µg	CFP75 CPZ30	4 x 50 Disks 4 x 50 Disks	67618 66298
Cefoperazone + Sulbactam	75/30 µg	SCF105	4 x 50 Disks	66734
Cefotaxime	5 µg 30 µg	COX5 CTX30	4 x 50 Disks 4 x 50 Disks	67718 66368

	DISK CONTENT	SYMBOL	PACK SIZE	Product code
Pipemidic Acid	20 µg	PIM20	4 x 50 Disks	68638
Piperacillin	30 µg	PIL30	4 x 50 Disks	68478
	100 µg	PIR100	4 x 50 Disks	67228
Piperacillin + Tazobactam	30/6 µg	PTZ36	4 x 50 Disks	67338
	100/10 µg	TZP110	4 x 50 Disks	67238
Polymixin	300 IU (50 µg)	PXB300	4 x 50 Disks	67248
Pristinamycin	15 µg	PTN15	4 x 50 Disks	67278
Quinupristin-Dalfopristin	15 µg	QDF15	4 x 50 Disks	67528
Rifampicin	5 µg	RIF5	4 x 50 Disks	66648
Spectinomycin	100 µg	SPT100	4 x 50 Disks	68798
Spiramycin	100 µg	SPN100	4 x 50 Disks	67378
Streptomycin	10 µg	SMN10	4 x 50 Disks	67418
Streptomycin (high load)	300 µg	HLS300	4 x 50 Disks	67608
	500 µg	STR500	4 x 50 Disks	67428
Sulphonamides	300 µg	SSS300	4 x 50 Disks	67578
	200 µg	SUL200	4 x 50 Disks	68408
Teicoplanin	30 µg	TEC30	4 x 50 Disks	68948
Temocillin	30 µg	TEM30	4 x 50 Disks	66068
Tetracycline	30 µg	TET30	4 x 50 Disks	67448
Ticarcillin	75 µg	TIC75	4 x 50 Disks	67458
Ticarcillin + Clavulanic Acid	75/10 µg	TCC85	4 x 50 Disks	67468
Tigecycline	15 µg	TGC15	4 x 50 Disks	67398
Tobramycin	10 µg	TMN10	4 x 50 Disks	67488
Trimethoprim + Sulfamethoxazole	1.25/23.75 µg	SXT25	4 x 50 Disks	68898
Trimethoprim	5 µg	TMP5	4 x 50 Disks	68888
Vancomycin	5 µg	VNC5	4 x 50 Disks	67828
	30 µg	VAN30	4 x 50 Disks	68928

4.2 Storage and handling requirements

Cartridges of disks must be stored in their containers at a temperature between +2°C and +8°C in a dry place.

The expiry date applies exclusively to disks contained in intact cartridges stored according to the manufacturer's instructions. The expiry date and batch number are indicated on each packaging (cartridge and container).

The stability of the disks, of open cartridges placed in distributors (preserved according to the recommendations with desiccants) was validated in routine conditions during 6 weeks, except for the antibiotic discs marked with the symbol , for which the stability in weeks is shown inside this symbol.

If the cartridge remains in the distributor after dispatch, it is necessary to preserve at +2-8°C in a dry place **with desiccants inside**.

5. WARNING AND PRECAUTIONS

For *in vitro* diagnostic use by professional user in a laboratory environment.

5.1 Health and Safety precautions

- This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves, eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
- Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.
- Always observe the current techniques and precautions concerning protection against microbiological hazards. After use, sterilize the cultures and all contaminated material.

5.2 Precautions related to the procedure

- Follow the instructions of the current guidelines (CLSI^(4,5), CA-SFM/EUCAST⁽⁶⁾, EUCAST⁽⁹⁾).
- Do not use the kit if the packaging of components is damaged.
- Container must be allowed to adjust to room temperature (18-30°C) during 20 minutes before opening. After applying the disks, return unused cartridges into their container to a temperature between +2°C and +8°C.
- Do not use disks after the expiry date. Do not use any cartridge of disks left at room temperature (18-30°C) for more than 8 hours without verifying an acceptable level of performance before continuing to use this cartridge⁽¹⁰⁾.

6. SPECIMENS

Disks must not be used for tests performed directly on biological samples.

Refer to the current guidelines (CLSI^(4,5), EUCAST⁽⁹⁾) defining preparation of the inoculum from a pure, fresh culture.

7. PROCEDURE

7.1 Materials required but not provided

- Disk dispenser: 6-7 disks Ref. # 50294
- 12-16 disks Ref. # 50295
- Culture media according to current guidelines (CLSI⁽⁴⁾, CA-SFM/EUCAST⁽⁶⁾, EUCAST⁽⁹⁾).
- Reagent.
- Bacterial strains for quality control • opacity control equivalent to the Mac Farland 0.5 standard.
- Laboratory equipment necessary for antibiotic susceptibility testing by the agar diffusion method.

7.2 Assay procedure

Refer to the instructions recommended by the CLSI^(4,5), the CA-SFM/EUCAST⁽⁶⁾ or the EUCAST⁽⁹⁾ for all steps of antibiotic susceptibility testing and interpretation of the results: the CLSI⁽⁴⁾, CA-SFM/EUCAST⁽⁶⁾, or EUCAST⁽⁹⁾ propose standardised techniques for preparation of the inoculum, inoculation of Petri dishes, the choice and arrangement of test disks, the incubation temperature and incubation time. Good laboratory practice should also be applied at all times.

7.3 Interpretation of the results

- Measure precisely the diameters of the zones of inhibition observed and refer to the critical diameters indicated by current guidelines (CLSI⁽⁵⁾, CA-SFM/EUCAST⁽⁶⁾, EUCAST⁽⁸⁾).
- A clinical category (intermediate resistant, susceptible or not susceptible...) is given to each micro-organism as a function of the observed diameter and the critical diameters for the antibiotic tested.
- These criteria of clinical categorization according to critical diameters are periodically revised by the CLSI⁽⁵⁾, CA-SFM/EUCAST⁽⁶⁾, EUCAST⁽⁸⁾.

8. TEST LIMITATION

- Inhibition zone measure can vary according to user. It can influence clinical categorization (resistant, intermediate, susceptible and non-susceptible). This incertitude must be considered as soon as interpretation results are closed to a categorization change.
- Antimicrobial agents other than indicated in table 2 may be used. Susceptibility tests employing these agents should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered as only qualitative until such time as interpretative zones have been established. All zone diameters should be recorded.
- The final interpretation, as for all laboratory interpretations, cannot be based on the results of one single test but on an overview of the clinical data and the biochemical, cytological and immunological results.
- The performances of the test depend not only on the activity of the disks, but also on factors such as the use of an appropriate inoculum and control strains, appropriate and previously tested culture media, an adequate storage.

	Disk content	Guidelines		Acceptable inhibition zone diameter (mm) Quality control limits			
		Internal	CA-SFM 2013 ⁽³⁾	<i>E. coli</i> ATCC® 25922™ [**ATCC® 35218™]	<i>S. aureus</i> ATCC® 25923™	<i>P. aeruginosa</i> ATCC® 27853™	<i>E. faecalis</i> ATCC® 29212™
Pipemicid acid	20 µg	✓		22-30			
Pristinamycin	15 µg		✓		27-32		
Spectinomycin	100 µg	✓		19-25			
Spiramycin	100 µg	✓			18-26		
Streptomycin	500 µg	✓			24-28		14-25
Sulfonamides	200 µg	✓			17-27		
Temocillin	30 µg	✓		17-23 22-28**			

For control limits (Internal standards):

Metronidazole 4 µg: *Clostridium perfringens* ATCC® 13124™ (≥ 15 mm), *Clostridium sporogenes* ATCC® 19404™ (≥ 15 mm).

10. BIBLIOGRAPHY REFERENCES

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6. CA-SFM/EUCAST: Comité de l'antibiogramme. French Society of Microbiology/ European Committee on Antimicrobial Susceptibility Testing. 2020 V1.1 APRIL.
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10. V. Loncle-Provot, E. Keller, M.O. Gourdin, M.L. Garrigues. Etude de la stabilité des disques antibiotiques dans les conditions d'utilisation en routine, 18th RICAI interdisciplinary meeting on anti-infectious chemotherapy, Paris, Dec.3/4 1998.

