

The antibiotic sensitivity discs

IVD For In-Vitro and professional use only

Store at (-20 to +8 °C)

INTENDED USE

For the Measurement of the Resistance and Sensitivity of the major microbial species to the antibiotic/chemotherapeutic agents.

INTRODUCTION

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem. Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area. The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

PRINCIPLE

The discs-diffusion test is based on the fact that for a given antibiotic, the size of the zone inhibition is inversely related to MIC (determined by dilution method) of the strain being tested when the test conditions are held constant. Antimicrobial susceptibility testing with discs is a simple and rapid method and provides a reproducible means of testing bacterial sensitivity to various antibiotics and chemotherapeutic agents.

MATERIALS PROVIDED

- Antibiotic discs.
- Dispenser.

MATERIALS REQUIRED BUT NOT PROVIDED

- Trypticase Soy Broth, 5 ml (or any other suitable broth).
- Mueller Hinton Agar, (MHA) Ph 7.3 ±0.1.
- Sterile Saline or Broth.

STORAGE AND STABILITY

All the sensitivity discs are stable at -20 C to +8 °C till the expiry date mentioned on individual label.

PRECAUTIONS

1. Allow the vials to come to room temperature before opening, to avoid condensation of moisture.
2. As soon as the application is over, tightly stopper and promptly transfer the vials to the refrigerator.

TEST PROCEDURE

1. Preparation of plates

Sterile Muller Hinton Agar, (pH 7.3 ±0.1) is poured into plates (petridishes) kept on a leveled surface. The depth of the medium should be approximately 4 mm. after the medium has solidified, dry the plates for 30 minutes in an incubator (35-37 °C) to remove excess moisture from the surface. While pouring into plates, 5% defibrinated sterile sheep blood should be aseptically added to MHA for testing Streptococci and other fastidious organisms.

2. Preparation of inoculum

- a) Use only pure culture for sensitivity testing. Perform Gram staining before preparing an inoculum.
- b) Select 4 to 5 similar colonies and transfer them into tube containing 5 ml of Trypticase Soy Broth (or similar broth) with the help of a wire loop.
- c) Incubate the broth culture at 35-37 °C for 2 to 5 hours to obtain moderate turbidity.
- d) Dilute the broth culture of actively growing organisms with sterile broth or saline to obtain a turbidity equivalent to that of Barium Sulphate Standard (equivalent to half the density of McFarland Standard No.1) which prepared by adding 0.5 ml of 1.175% BaCl₂ 2H₂O solution to 99.5 ml of 0.36N H₂SO₄. overnight cultures of aerobes/non-fastidious organisms generally have too much growth to be used undiluted. When time does not permit for the development of a sufficiently turbid broth culture, colonies can be suspended directly into a small volume of saline or broth which is then further up to the turbidity equivalent to half that of McFarland Standard No.1 . in order to prevent further growth the diluted and standardized inoculums should not be allowed to stand longer than 15 to 20 minutes before the plates are inoculated.

3. Inoculation :

- a) Dip a sterile cotton swab into the appropriately diluted culture inoculum and rotate it while pressing against the upper inside wall of the tube, above the fluid level to remove excess inoculum.
- b) Streak the agar surface of the plate in three directions, tuning the plate by 60 between each streaking.
- c) Replace the lid of the Petri dish and keep it at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculum. Confluent growth is desirable for accurate results.

4. Application of sensitivity-discs

Remove one sensitivity disc from the cartridge with the help of a flamed forceps and carefully place it on the surface of the medium.

Finally, press it tightly with the forceps to make complete contact with the surface of the medium. Allow the plate to stand at room temperature for 30 minutes (prediffusion time) before proceeding for next step.

5. Incubation

Incubate the plates at 35-37 °C for 16 to 18 hours.

6. Reading the zones

Measure the diameter of the zone of inhibition at the end of the incubation period. If plates are showing proper growth, they may be read after 6 to 8 hours in clinically urgent situations. Measure only those zones that are showing complete inhibition and record the zone diameter to the nearest millimeter. If only isolated colonies grow instead of confluent growth, the inoculum is too light and the test should be repeated.

INTERPRETATION OF RESULTS

Although there is some correlation between the size of the zone of inhibition and susceptibility of the organisms to the antibiotic, the former is a function of many variables e.g. density of the inoculum, depth of the medium, diffusibility of antibiotic etc.

The size of the inhibition zone at which organisms is considered Resistant; intermediate or sensitive is given in the zone size interpretative chart as a part of this literature.

Zone diameters Interpretive Chart

Serial No.	Antimicrobial Agent	Code	Disc Content	Test organisms	Zone Diameter Nearest Whole mm		
					Resistance ≤	Intermediate	Sensitive ≥
1.	Amikacin	AK	30 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> , <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp	14	15-16	17
2.	Amoxycillin	AX	10 µg	All organisms	13	14-17	18
3.	Amoxycillin/ Clavulanic acid	AC	20±10 µg	<i>Enterobacteriaceae</i>	13	14-17	18
				<i>Staphylococcus</i> spp. <i>Haemophilus</i> spp. <i>H.parainfluenzae</i> .	19	-----	20
4.	Ampicillin	AP	10 µg	<i>Enterobacteriaceae</i> <i>Vibrio cholera</i>	13	14-16	17
				<i>Staphylococcus</i> spp.	28	-----	29
				<i>Enterococcus</i> spp.	16	-----	17
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	18	19-21	22
				<i>Streptococcus</i> spp.(other than <i>Streptococcus pneumoniae</i>) (Beta group)	-----	-----	24
5.	Ampicillin/ Sulbactam	AS	10+10 µg	<i>Enterobacteriaceae</i> <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	11	12-14	15
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	19	---	20
6.	Azithromycin	AZ	15 µg	<i>Staphylococcus</i> spp. <i>Streptococcus</i> spp.	13	14-17	18
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	---	---	12
				<i>Neisseria meningitides</i>	---	---	20
							22
7.	Azterionam	AT	30 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp.	15	16-21	22
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	---	---	26
8.	Cefaclor	CG	30 µg	<i>Enterobacteriaceae</i> <i>Staphylococcus</i> spp.	14	15-17	18
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	16	17-19	20
9.	Cefadroxile	CD	30 µg	All organisms	14	15-17	18
10.	Cefazolin	CF	30 µg	<i>Enterobacteriaceae</i> <i>Staphylococcus</i> spp.	14	15-17	18
11.	Cefdinir	CN	5 µg	<i>Enterobacteriaceae</i> <i>Staphylococcus</i> spp.	16	17-19	20
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	---	---	20
12.	Cefixime	FX	5 µg	<i>Enterobacteriaceae</i>	15	16-18	19
				<i>Haemophilus</i> spp.	---	---	21
				<i>Neisseria meningitides</i>	---	---	31
13.	Cefoperazone	PZ	75 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Staphylococcus</i> spp.	15	16-20	21
14.	Cefoperazone /sulbactam	CS	75+30 µg	<i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	15	16-20	21
15.	Cefotaxime	CX	30 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	14	15-22	23
				<i>Haemophilus</i> spp. <i>H.parainfluenzae</i>	---	---	26
				<i>Neisseria gonorrhoeae</i> .	---	---	31
				<i>Staphylococcus</i> spp.	25	26-27	28


				(Other than <i>Streptococcus pneumoniae</i>) If viridians group			
				if beta group	---	---	24
				<i>Neisseria meningitides</i>	---	---	34
16.	Cefpirome	CE	30 µg	<i>Enterobacteriaceae</i>	24	26-27	28
				<i>Acinetobacter</i> spp.	20	21-23	24
				<i>Pseudomonas aeruginosa</i> .			
17.	Cefpodoxime	CO	10 µg	<i>Enterobacteriaceae</i>	17	18-20	21
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.	----	---	21
				<i>H.parainfluenza</i>			
18.	Cefprozil	FP	30 µg	<i>Enterobacteriaceae</i>	14	15-17	18
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.			
				<i>H.parainfluenza</i>			
19.	Ceftazidime	CZ	30 µg	<i>Enterobacteriaceae</i>	14	15-17	18
				<i>Pseudomonas aeruginosa</i> .			
				<i>Acinetobacter</i> spp.			
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.	---	---	26
				<i>H.parainfluenza</i>			
				<i>Burkholderia cepacia</i>	17	18-20	21
				<i>Neisseria gonorrhoeae</i> .	---	---	31
20.	Ceftizoxime	FO	30 µg	<i>Enterobacteriaceae</i>	14	15-19	20
				<i>Pseudomonas aeruginosa</i> .			
				<i>Acinetobacter</i> spp.			
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.	---	---	26
				<i>H.parainfluenza</i>			
				<i>Neisseria gonorrhoeae</i> .	---	---	38
21.	Ceftriaxone	FR	30 µg	<i>Enterobacteriaceae</i>	13	14-20	21
				<i>Pseudomonas aeruginosa</i> .			
				<i>Acinetobacter</i> spp.			
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.	---	---	26
				<i>H.parainfluenza</i>			
				<i>Neisseria gonorrhoeae</i> .	---	---	35
				<i>Streptococcus</i> spp (Other than <i>Streptococcus pneumoniae</i>) If viridians group	24	25-26	27
				if beta group	---	---	24
				<i>Neisseria meningitides</i>	---	---	34
22.	Cefuroxime Sodium	CR	30 µg	<i>Enterobacteriaceae</i>	14	15-17	18
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.	16	17-19	20
				<i>H.parainfluenza</i>			
				<i>Neisseria gonorrhoeae</i> .	25	26-30	31
23.	Cephalexin	CP	30 µg	All Organisms	14	15-17	18
24.	Cephaloridine	CH	30 µg	All Organisms	11	12-15	16
25.	Cephalothin	CA	30 µg	<i>Enterobacteriaceae</i>	14	15-17	18
				<i>Staphylococcus</i> spp.			
26.	Chloramphenicol	CK	30 µg	<i>Enterobacteriaceae</i>	12	13-17	18
				<i>Staphylococcus</i> spp.			
				<i>Enterococcus</i> spp.			
				<i>Vibrio cholerae</i>			
				<i>Haemophilus</i> spp.	25	26-28	29
				<i>H.parainfluenza</i>			
				<i>Neisseria meningitides</i>	19	20-25	26
				<i>Streptococcus pneumoniae</i>	20	---	21
				<i>Streptococcus</i> spp (Other than <i>Streptococcus pneumoniae</i>)	17	18-20	21
27.	Ciprofloxacin	CI	5 µg	<i>Enterobacteriaceae</i>	15	16-20	21
				<i>Pseudomonas aeruginosa</i> .			
				<i>Acinetobacter</i> spp.			
				<i>Staphylococcus</i> spp.			
				<i>Enterococcus</i> spp.			
				<i>Haemophilus</i> spp.	---	---	21
				<i>H.parainfluenza</i>			
				<i>Neisseria gonorrhoeae</i>	27	28-40	41
				<i>Neisseria meningitides</i>	32	33-34	35
28.	Clarithromycin	CL	15 µg	<i>Staphylococcus</i> spp.	13	14-17	18
				<i>Haemophilus</i> spp.	10	11-12	13
				<i>H.parainfluenza</i>			
				<i>Streptococcus</i> spp.	16	17-20	21
29.	Clindamycin	CM	2 µg	<i>Staphylococcus</i> spp.	14	15-20	21
				<i>Streptococcus</i> spp.	15	16-18	19
30.	Claxacillin	CV	5 µg	All organisms	11	12-13	14
31.	Co-Trimoxazole	CT	25 µg	<i>Enterobacteriaceae</i>	10	11-15	16
				<i>Acinetobacter</i> spp.			
				<i>Staphylococcus</i> spp.			
				<i>Haemophilus</i> spp.			
				<i>H.parainfluenza</i>			
				<i>Vibrio cholerae</i>			
				<i>Burkholderia cepacia</i>			
				<i>Stenotrophomonas maltophilia</i>			
				<i>Streptococcus pneumoniae</i>	15	16-18	19












				<i>Neisseria meningitides</i>	25	26-29	30
32.	Doxycycline	DO	30 µg	<i>Enterobacteriaceae</i>	10	11-13	14
				<i>Acinetobacter</i> spp.	9	10-12	13
				<i>Staphylococcus</i> spp.	12	13-15	16
				<i>Enterococcus</i> spp.			
33.	Erythromycin	ER	15 µg	<i>Staphylococcus</i> spp. <i>Enterococcus</i> spp.	13	14-22	23
34.	Furazolidone	FZ	100 µg	All organisms	14	15-16	17
35.	Gatifloxacin	GF	5 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Enterococcus</i> spp.	14	15-17	18
				<i>Staphylococcus</i> spp.	19	20-22	23
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	18
				<i>Neisseria gonorrhoeae</i>	33	34-37	38
				<i>Streptococcus</i> spp.	17	18-20	21
36.	Gentamicin	GM	10 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	12	13-14	15
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	16
37.	Imipenem \ Cilastain	IS	10+10 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	13	14-15	16
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	16
38.	Kanamycin	KA	30 µg	<i>Enterobacteriaceae</i> <i>Staphylococcus</i> spp.	13	14-17	18
39.	Levofloxacin	LV	5 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> . <i>Acinetobacter</i> spp. <i>Enterococcus</i> spp. <i>Streptococcus</i> spp. <i>Stenotrophomonas maltophilia</i> .	13	14-16	17
				<i>Staphylococcus</i> spp.	15	16-18	19
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	17
40.	Linomycin	LN	15 µg	All organisms	13	14-17	18
41.	Linezolid	LI	30 µg	<i>Staphylococcus</i> spp.	---	---	21
				<i>Enterococcus</i> spp.	20	21-22	23
				<i>Streptococcus</i> spp.	---	---	21
42.	Lemefloxacin	LF	5 µg	<i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus</i> spp.	18	19-21	22
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	22
				<i>Neisseria gonorrhoeae</i>	26	27-37	38
43.	Meropenem	MR	10 µg	<i>Enterobacteriaceae</i> <i>Pseudomonas aeruginosa</i> <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp.	13	14-15	16
				<i>Burkholderia cepacia</i>	15	16-19	20
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	20
				<i>Neisseria meningitides</i>	---	---	30
44.	Minocycline	MN	30 µg	<i>Enterobacteriaceae</i> <i>Acinetobacter</i> spp.	12	13-15	16
				<i>Staphylococcus</i> spp. <i>Enterococcus</i> spp. <i>Burkholderia cepacia</i> <i>Stenotrophomonas maltophilia</i> .	14	15-18	19
				<i>Neisseria meningitides</i>	---	---	26
45.	Moxifloxacin	MF	5 µg	<i>Staphylococcus</i> spp.	20	21-23	24
				<i>Streptococcus</i> spp.	14	15-17	18
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i>	---	---	18
46.	Nalidixic acid	NA	30 µg	<i>Enterobacteriaceae</i> <i>Neisseria meningitides</i>	13 25	14-18 ---	19 26
47.	Nitrofurantoin	FU	300 µg	<i>Enterobacteriaceae</i> . <i>Staphylococcus</i> spp. <i>Enterococcus</i> spp.	14	15-16	17
48.	Norfloxacin	NF	10 µg	<i>Enterobacteriaceae</i> . <i>Pseudomonas aeruginosa</i> . <i>Staphylococcus</i> spp. <i>Enterococcus</i> spp.	12	13-16	17
49.	Ofloxacin	OF	5 µg	<i>Enterobacteriaceae</i> . <i>Pseudomonas aeruginosa</i> . <i>Streptococcus</i> spp.	12	13-15	16
				<i>Staphylococcus</i> spp.	14	15-17	18
				<i>Haemophilus</i> spp. <i>H.parainfluenza</i> .	---	---	16
				<i>Neisseria gonorrhoeae</i> .	24	25-30	31
50.	Pefloxacin	PF	5 µg	All organisms	12	13-16	17
51.	Penicillin-G	PG	10 IU	<i>Staphylococcus</i> spp.	28	---	29
				<i>Enterococcus</i> spp.	14	---	15
				<i>Neisseria gonorrhoeae</i> .	26	27-46	47

				<i>Streptococcus</i> spp. (Other than <i>Streptococcus pneumoniae</i>) Beta group	---	---	24
52.	Piperacillin	PC	100 µg	<i>Enterobacteriaceae.</i> <i>Acinetobacter</i> spp. <i>Pseudomonas aeruginosa</i>	17	18-20	21
					17	---	18
53.	Piperacillin/ Tazobacum	PT	100+10 µg	<i>Enterobacteriaceae.</i> <i>Acinetobacter</i> spp. <i>Pseudomonas aeruginosa.</i> <i>Staphylococcus</i> spp.	17	18-20	21
					17	---	18
54.	Rifampin	RN	5 µg	<i>Staphylococcus</i> spp. <i>Enterococcus</i> spp. <i>Haemophilus</i> spp. <i>H.parainfluenzae.</i>	16	17-19	20
				<i>Streptococcus pneumoniae</i>	16	17-18	19
				<i>Neisseria meningitides.</i>	19	20-24	25
55.	Roxithromycin	RO	30 µg	All organisms	9	10-20	21
56.	Sparfloxacin	SP	5 µg	<i>Staphylococcus</i> spp. <i>Streptococcus pneumoniae</i>	15	16-18	19
57.	Streptomycin	ST	10 µg	<i>Enterobacteriaceae.</i>	11	12-14	15
58.	Sulfadiazine	SD	300 µg	<i>Enterobacteriaceae.</i> <i>Staphylococcus</i> spp. <i>Vibrio cholera.</i>	12	13-16	17
59.	Teicoplanin	TP	30 µg	<i>Staphylococcus</i> spp.	10	11-13	14
60.	Tetracycline	TE	30 µg	<i>Enterobacteriaceae.</i> <i>Acinetobacter</i> spp. <i>Staphylococcus</i> spp. <i>Enterococcus</i> spp. <i>Vibrio cholera.</i>	11	12-14	15
					14	15-18	19
				<i>Haemophilus</i> spp. <i>H.parainfluenzae.</i>	25	26-28	29
				<i>Neisseria gonorrhoeae</i>	30	31-37	38
				<i>Streptococcus</i> spp.	18	19-22	23
61.	Ticarcillin/ Clavulanic acid	TC	75+2.5 µg	<i>Enterobacteriaceae.</i> <i>Acinetobacter</i> spp. <i>Pseudomonas aeruginosa.</i> <i>Staphylococcus</i> spp.	14	15-19	20
					14	---	15
					22	---	23
62.	Tobramycin	TO	10 µg	<i>Enterobacteriaceae.</i> <i>Acinetobacter</i> spp. <i>Pseudomonas aeruginosa.</i> <i>Staphylococcus</i> spp.	12	13-14	15
63.	Trimethoprim	TR	5 µg	<i>Enterobacteriaceae.</i> <i>Staphylococcus</i> spp.	10	11-15	16
64.	Vancomycin	VM	30 µg	<i>Staphylococcus</i> spp. <i>Enterococcus</i> spp. <i>Streptococcus</i> spp.	14	15-16	15
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PPI918A01
Rev B (03.11.2015)

	Product Reference No.		For in-vitro diagnostic use.
	Caution.		Store at
	Read product insert before use.		Number of tests in the pack.
	Lot (batch) number.		Manufacturer.
	Expiry date.		Manufacturer telephone number.
	Manufacturer fax number.		