

Determination of minimum inhibitory concentrations

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Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. MBC determinations are undertaken less frequently and their major use has been reserved for isolates from the blood of patients with endocarditis. Standardized methods for determining MICs and MBCs are described in this paper. Like all standardized procedures, the method must be adhered to and may not be adapted by the user. The method gives information on the storage of standard antibiotic powder, preparation of stock antibiotic solutions, media, preparation of inocula, incubation conditions, and reading and interpretation of results. Tables giving expected MIC ranges for control NCTC and ATCC strains are also supplied.

Introduction

Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate, for example when determining the susceptibility of coagulase-negative staphylococci to teicoplanin.

The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1 mg/L as required. The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after overnight incubation (this period is extended for organisms such as anaerobes, which require prolonged incubation for growth).

The method described below is an amended version of the procedure described in the BSAC Guide to Sensitivity Testing¹ and can be adapted for determining the minimum bactericidal concentration (MBC) of an antimicrobial for an organism by substituting IsoSensitest agar (ISA; Oxoid, Basingstoke, UK) with IsoSensitest broth (ISTB; Oxoid) and then subculturing to drug-free media or can be truncated for use as a 'breakpoint' method. However, if the

method is adapted, the control strains cited below may not act as adequate controls for the concentration of antibiotic contained within prepared plates.

1. Antibiotic stock solutions: general considerations

1.1 Obtain standard powder from the pharmaceutical company or a reputable supplier such as Sigma (Poole, Dorset, UK).

1.2 Obtain information from the supplier regarding expiry date, potency, solubility, stability as a powder and in solution, storage conditions and any relevant COSHH (Control of Substances Hazardous to Health) information.

1.3 Always prepare stock solutions following the manufacturer's recommendations.

1.4 Freeze and thaw stock solutions only once and then discard them. Table I shows the suppliers, solvent, diluents and storage conditions for antibiotics.

2. Preparation of antibiotic stock solutions

2.1 Choose a suitable range of antibiotic concentrations for the organisms to be tested (see suggested ranges in Table II).

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Table I. Preparation and storage of antibiotic solutions (stored solutions should contain ≥ 1000 mg/L)

Antibiotic	Solvent	Diluent	4°C	-20°C	-70°C	Storage of powder	Supplier ^a
14 hydroxylarithmeticromycin	methanol	water	-	-	-	+4°C; protect from light and moisture	Abbott Laboratories
Amikacin (base)	water	water	7 days	1 month	-	+4-25°C; protect from moisture and light	Bristol Myers Squibb
Amoxicillin (trihydrate)	DMSO or ^b	water	7 days	unstable	30 days	+4°C; protect from light and moisture	GlaxoSmithKline
Ampicillin (trihydrate)	^c	water	7 days	unstable	30 days	+4°C; protect from light and moisture	GlaxoSmithKline
Azithromycin (dihydrate)	^b	water	-	-	-	+4-25°C; protect from moisture and light	Pfizer
Aztreonam (anhydrous crystalline B form)	water	water	1 day	3 months	-	+4°C; protect from light and moisture	Bristol Myers Squibb
Carbenicillin (disodium)	water	water	-	-	-	+4°C; protect from light and moisture	GlaxoSmithKline
Cefaclor	water	water	-	-	-	+4°C; protect from light and moisture	Eli Lilly & Co Ltd
Cefepime (dihydrochloride)	^b	water	-	-	-	+4°C; protect from light and moisture	Bristol Myers Squibb
Cefixime	water	water	-	-	-	2-8°C; protect from moisture and light	Wyeth Laboratories
Cefotaxime (sodium)	water	water	10 days	6 months	6 months	+4-25°C; protect from moisture and light	Aventis Pharma
Cefoxitin (sodium)	water	water	-	6 months	-	+4-25°C; protect from moisture and light	Merck Sharpe & Dohme Ltd
Cefpirome (sulphate)	water	water	-	-	-	2-8°C; protect from moisture and light	Aventis Pharma
Cefpodoxime (sodium)	^b	water	-	-	-	2-8°C; protect from moisture and light	Aventis Pharma
Ceftazidime (pentahydrate)	water	water	1 day	3 months	-	+4-25°C; protect from moisture and light	GlaxoSmithKline
Ceftizoxime (sodium)	water	water	7 days	-	-	+4-25°C; protect from moisture and light	GlaxoSmithKline
Ceftriaxone (disodium)	water	water	-	-	-	2-8°C; protect from moisture and light	Roche Products Ltd
Cefuroxime (sodium)	water	water	3 days	30 days	-	+4°C; protect from light and moisture	GlaxoSmithKline
Cephalexin (hydrate)	water	water	7 days	-	-	+4°C; protect from light and moisture	GlaxoSmithKline
Cephadrine	^c	water	1 day	-	-	+4°C; protect from light and moisture	Bristol Myers Squibb
Chloramphenicol	water	water	2 weeks	3 months	3 months	+4°C; protect from light and moisture	Sigma
Ciprofloxacin (hydrochloride monohydrate)	water	water	2 weeks	3 months	3 months	+4-25°C; protect from moisture and light	Bayer
Clarithromycin	DMSO ^d	water	-	-	-	15-30°C; protect from light and moisture	Abbott Laboratories
Clavulanate (acid)	^d	water	1-5 days	unsuitable	4 weeks	2-8°C; protect from moisture and light	GlaxoSmithKline
Clindamycin (hydrochloride)	water	water	-	-	-	+4°C; protect from light and moisture	Sigma
Cloxacillin (sodium monohydrate)	water	water	-	-	-	15-30°C; protect from light and moisture	GlaxoSmithKline
Colistin (sulphate)	water	water	-	-	-	2-8°C; protect from moisture and light	Pharmax
Doxycycline (hydrochloride)	water	water	-	-	-	2-8°C; protect from moisture and light	Pfizer
Erythromycin (base)	^c	water	1 week	-	-	+4°C; protect from light and moisture	Abbott Laboratories
Flucloxacillin (sodium)	water	water	-	-	-	2-8°C; protect from moisture and light	GlaxoSmithKline
Fosfomycin (calcium)	water	water	-	-	-	2-8°C; protect from moisture and light	Pharmax
Fusidic acid (sodium)	^c	water	-	-	-	+4-25°C; protect from moisture and light	Leo Laboratories
Gatifloxacin	^e	water	-	-	-	+4°C; protect from light and moisture	Grunenthal
Gemifloxacin (base)	methanol	water	-	-	-	+4°C; protect from light and moisture	GlaxoSmithKline
Gentamicin (sulphate)	water	water	6 months	NR	NR	+4-25°C; protect from moisture and light	Aventis Pharma
Grepafloxacin (hydrochloride)	^e	water	-	-	-	+4°C; protect from light and moisture	GlaxoSmithKline

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<i>f</i>	<i>h</i>	<i>g</i>					
Telithromycin							Aventis Pharma
Imipenem (monohydrate)							Merck Sharpe & Dohme Ltd
Kanamycin (monosulphate)	water	water	1 day	NR	1 month		Sanofi Winthrop
Levofloxacin (hemihydrate)	water	water	-	-	-		Aventis Pharma
Linezolid	water	water	-	-	-		Pharmacia & Upjohn Ltd
Mecillinam	water	water	-	-	-		Leo Laboratories
Meropenem (trihydrate)	water	water	-	-	-		Zeneca Pharma
Methicillin (sodium)	water	water	-	-	-		GlaxoSmithKline
Metronidazole	water	water	-	-	-		Aventis Pharma
Mezlocillin	water	water	1 week	1 month	4 months		Bayer
Moxifloxacin (hydrochloride)	water	water	-	-	-		Bayer
Mupirocin (lithium)	water	water	-	-	-		GlaxoSmithKline
Nalidixic acid	water	water	-	-	-		Sanofi Winthrop
Netilmicin (sulphate)	water	water	6 months	6 months	6 months		Schering Plough
Nitrofurantoin	DMF	DMF	-	-	-		Procter & Gamble
Norfloxacin	water	water	-	-	-		Merck Sharpe & Dohme Ltd
Ofloxacin	water	water	-	-	-		Aventis Pharma
Oxacillin (sodium)	water	water	-	-	-		GlaxoSmithKline
Penicillin (benzyl)[potassium]	water	water	-	1 month	1 month		GlaxoSmithKline
Piperacillin (sodium)	water	water	2 days	1 month	-		Wyeth Laboratories
Quinupristin/dalfopristin	water	water	-	1 month	-		Aventis Pharma
Rifampicin (crystalline)	DMSO	water	1 month	1 month	-		Aventis Pharma
Roxithromycin	water	water	-	-	-		Aventis Pharma
Sparfloxacin	water	water	-	-	-		Aventis Pharma
Spectinomycin	water	water	-	-	-		Pharmacia & Upjohn Ltd
(dihydrochloride pentahydrate)							
Streptomycin (sulphate)	water	water	-	-	-		Medeva Pharma Ltd
Sulphamethoxazole (free acid)	water	water	1 month	6 months	2 years		GlaxoSmithKline
Tazobactam (sodium salt)	water	water	-	-	-		Wyeth Laboratories
Teicoplanin	water	water	-	-	-		Aventis Pharma
Tetracycline (hydrochloride)	water	water	-	NR ⁱ	NR ⁱ		Wyeth Laboratories
Ticarcillin (sodium)	water	water	1 week	1 month	-		GlaxoSmithKline
Tobramycin (sulphate)	water	water	1 week	3 months	-		Eli Lilly & Co Ltd
Trimethoprim (base)	water	water	1 month	6 months	2 years		GlaxoSmithKline
Vancomycin (hydrochloride)	water	water	1 week	3 months	-		Eli Lilly & Co Ltd

^aMany agents are available from Sigma, Poole, UK.

^bSaturated NaHCO₃ solution.

^cEthanol.

^dPhosphate buffer (0.1 M, pH 6).

^eWater and 0.1 M NaOH dropwise to dissolve.

^fWater (1 mL) + 10 µL glacial acetic acid.

^gPhosphate buffer (0.07 M, pH 8).

^h1 M MOPS pH 6.8 buffer.

ⁱPrecipitation on freezing.

NR = not recommended; DMF = dimethylformamide; DMSO = dimethylsulphoxide. All solutions should be placed in glass containers.

Table II. Suggested ranges for MIC determinations (mg/L)

Antibiotic	Enterobacteriaceae	<i>Pseudomonas</i> spp.	<i>Haemophilus</i> spp.	<i>Neisseria</i> spp.	<i>B. fragilis</i>	Staphylococci	Haemolytic streptococci	Enterococci	Pneumococci
Amikacin	0.03-128	0.06-128	0.12-16	0.5-16	-	0.008-128	1-128	1-128	1-128
Amoxicillin	0.25-128	-	0.06-128	0.004-32	1-128	0.03-128	0.008-0.12	0.12-128	0.008-4
Ampicillin	0.25-128	-	0.06-128	0.004-32	1-128	0.03-128	0.008-0.12	0.12-128	0.008-4
Azithromycin	0.25-128	-	-	-	-	-	-	-	-
Azlocillin	0.25-128	0.5-512	0.03-2	0.004-8	1-16	0.06-128	-	-	-
Aztreonam	0.004-128	0.5-128	0.015-2	0.015-2	8-128	>128	-	-	-
Cefaclor	-	-	0.5-128	-	-	-	-	-	0.25-64
Cefixime	0.03-128	-	0.008-0.12	0.002-1	8-128	4-64	0.03-0.5	8-128	0.12-16
Cefotaxime	0.004-128	0.5-128	0.004-0.5	0.004-0.5	0.5-128	0.5-128	-	-	-
Cefoxitin	0.5-128	-	1-8	0.06-8	2-128	1-32	-	-	-
Cefpirome	0.008-32	0.25-128	0.008-0.5	0.001-0.12	4-128	0.06-128	0.004-0.12	1-128	0.008-1
Cefpodoxime	0.06-128	0.25-128	0.06-0.5	0.002-0.06	8-128	1-128	0.015-0.12	1-128	0.03-4
Ceftazidime	0.004-128	0.25-128	0.015-0.5	0.004-0.5	4-128	2-128	0.03-1	0.12-128	0.03-32
Ceftizoxime	0.004-128	-	0.008-0.25	0.004-0.015	0.5-128	1-128	-	-	-
Ceftriaxone	0.001-128	0.5-128	0.001-0.06	0.001-0.06	2-128	0.25-128	0.008-0.12	0.004-128	0.004-16
Cefuroxime	0.03-128	-	0.25-16	0.008-1	1-128	0.25-64	0.008-0.12	2-128	0.015-8
Cephalexin	0.25-128	-	1-128	-	4-128	0.5-128	-	-	-
Cephadrine	0.25-128	-	1-128	-	1-128	0.25-128	-	-	-
Chloramphenicol	0.25-128	-	0.06-128	0.06-8	1-8	2-16	1-16	1-128	1-16
Ciprofloxacin	0.004-128	0.015-128	0.002-0.06	0.001-0.12	2-8	0.06-128	0.12-4	0.25-128	0.25-128
Clarithromycin	-	-	1-32	0.015-1	0.03-2	0.03-128	0.015-16	0.03-128	0.03-128
Co-amoxiclav ^o	0.5-128	-	0.03-128	0.004-32	0.5-128	0.008-16	0.008-0.12	0.12-16	0.008-4
Clindamycin	-	-	-	-	0.015-2	0.03-8	-	-	-
Colistin	0.5-128	0.5-64	-	-	-	-	-	-	-
Quinupristin/dalfopristin	-	-	-	-	4-32	0.12-16	0.12-1	0.25-8	0.12-32
Doxycycline	-	-	0.03-128	0.25-16	-	0.06-128	-	-	-
Erythromycin	-	-	0.25-128	0.03-0.5	-	0.06-128	0.06-8	0.25-128	0.06-128
Fusidic acid	-	-	-	-	0.25-128	0.03-128	-	-	-
Gatifloxacin	-	-	-	0.001-0.12	-	-	-	-	-
Gemifloxacin	-	-	-	0.001-0.12	-	-	-	-	-
Gentamicin	0.03-128	0.06-128	0.12-16	0.5-16	-	0.008-128	-	0.5-2048	-
Grepafloxacin	-	-	0.002-0.06	0.001-0.12	-	-	-	-	-
Telithromycin	-	-	0.25-8	0.002-0.5	0.03-8	0.03-128	0.001-0.25	0.015-4	0.004-1
Imipenem	0.06-4	0.06-16	0.25-4	0.004-0.25	0.015-4	0.03-128	0.002-0.25	0.25-128	0.002-0.25
Levofloxacin	-	-	-	0.001-0.12	-	-	-	-	0.5-32

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Linezolid	0.03-128	0.12-16	0.007-1	-	1-4	0.12-8	0.25-8	0.25-8	0.5-8
Mecillinam	0.03-128	-	-	-	-	-	-	-	-
Meropenem	0.015-4	0.015-16	0.015-0.25	0.002-0.03	0.03-32	0.015-128	0.002-0.06	0.004-128	0.002-16
Methicillin	-	-	-	-	0.06-32	0.12-128	-	-	-
Metronidazole	-	-	-	-	1-128	-	-	-	-
Mezlocillin	0.25-128	0.5-512	-	-	-	0.12-128	-	-	-
Moxifloxacin	-	-	-	0.001-0.12	-	-	-	-	-
Mupirocin	-	-	-	-	-	0.06-1024	-	-	-
Nalidixic acid	1-128	32-128	0.015-2	0.5-8	32-64	16-128	-	-	-
Netilmicin	0.03-128	0.06-128	0.12-16	0.5-16	-	0.008-128	-	-	-
Ofloxacin	0.06-128	0.25-8	0.015-2	0.001-0.06	1-8	0.12-128	-	1-128	1-128
Oxacillin	-	-	-	-	-	0.12-128	-	-	-
Penicillin	-	-	-	0.004-32	4-128	0.015-128	0.004-0.06	0.5-128	0.015-4
Piperacillin	0.25-128	0.5-512	0.004-128	0.015-32	0.25-128	0.25-128	-	-	-
Rifampicin	-	-	-	0.25-2	-	0.004-128	-	-	-
Roxithromycin	-	-	2-32	0.015-2	0.12-16	0.03-128	0.015-16	0.03-128	0.03-128
Sparfloxacin	0.008-128	0.12-16	0.004-0.03	0.001-0.12	0.12-1	0.06-0.25	0.12-1	0.25-128	0.12-128
Spectinomycin	-	-	-	4-64	-	-	-	-	-
Sulphamethoxazole	4-128	-	0.5-32	0.25-8	-	-	-	-	-
Teicoplanin	-	-	-	-	-	0.06-32	-	0.5-2048	-
Tetracycline	0.25-128	-	0.06-128	-	-	0.06-128	-	-	-
Ticarcillin	0.25-128	0.5-512	0.06-128	-	4-128	0.5-128	-	-	-
Tobramycin	0.03-128	0.06-128	-	0.5-16	-	0.008-128	-	-	-
Trimethoprim	0.03-128	-	0.015-16	-	-	0.03-8	-	-	-
Vancomycin	-	-	-	-	-	0.06-32	0.12-1	0.12-128	0.12-1

*Ratio of one part clavulamic acid:two parts amoxicillin.

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