

## Determination of minimum inhibitory concentrations

Jennifer M. Andrews\*

Department of Microbiology, City Hospital NHS Trust, Birmingham B18 7QH, UK

**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 Testing<sup>1</sup> 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).

---

\*Tel: +44-121-507-5693; Fax: +44-121-551-7763; E-mail: Jenny.Andrews@cityhospbham.wmids.nhs.uk

---

**Table I.** Preparation and storage of antibiotic solutions (stored solutions should contain  $\geq 1000$  mg/L)

Antibiotic	Solvent	Diluent	4°C	-20°C	-70°C	Storage of powder	Supplier <sup>a</sup>
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 <sup>b</sup>	water	7 days	unstable	30 days	+4°C; protect from light and moisture	GlaxoSmithKline
Ampicillin (trihydrate)	<sup>c</sup>	water	7 days	unstable	30 days	+4°C; protect from light and moisture	GlaxoSmithKline
Azithromycin (dihydrate)	<sup>b</sup>	water	-	-	-	+4-25°C; protect from moisture and light	Pfizer
Aztreonam (anhydrous crystalline B form)	<sup>b</sup>	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)	<sup>b</sup>	water	-	-	-	+4°C; protect from light and moisture	Bristol Myers Squibb
Cefixime	<sup>b</sup>	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)	water	water	-	-	-	2-8°C; protect from moisture and light	Aventis Pharma
Ceftazidime (pentahydrate)	<sup>b</sup>	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
Cephradine	<sup>c</sup>	water	1 day	-	-	+4°C; protect from light and moisture	Bristol Myers Squibb
Chloramphenicol	<sup>c</sup>	water	-	-	-	+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 <sup>d</sup>	water	-	-	-	15-30°C; protect from light and moisture	Abbott Laboratories
Clavulanate (acid)	<sup>d</sup>	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)	<sup>c</sup>	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)	<sup>c</sup>	water	-	-	-	+4-25°C; protect from moisture and light	Leo Laboratories
Gatifloxacin	<sup>e</sup>	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)	<sup>e</sup>	water	-	-	-	+4°C; protect from light and moisture	GlaxoSmithKline

Determination of MICs

<i>f</i>	<i>h</i>	<i>g</i>	<i>h</i>	<i>f</i>	<i>h</i>	<i>g</i>	<i>h</i>
Telithromycin	water	water	1 day	NR	1 month	+4°C; protect from light and moisture	Aventis Pharma
Imipenem (monohydrate)	water	water	1 day	NR	1 month	15–30°C; protect from light and moisture	Merck Sharpe & Dohme Ltd
Kanamycin (monosulphate)	water	water	–	–	–	+4°C; protect from light and moisture	Sanofi Winthrop
Levofloxacin (hemihydrate)	water	water	–	–	–	+4°C; protect from light and moisture	Aventis Pharma
Linezolid	water	water	–	–	–	+4°C; protect from light and moisture	Pharmacia & Upjohn Ltd
Mecillinam	water	water	–	–	–	+4°C; protect from light and moisture	Leo Laboratories
Meropenem (trihydrate)	water	water	–	–	–	+4°C; protect from light and moisture	Zeneca Pharma
Methicillin (sodium)	water	water	–	–	–	+4°C; protect from light and moisture	GlaxoSmithKline
Metronidazole	water	water	–	–	–	+4–25°C; protect from moisture and light	Aventis Pharma
Mezlocillin	water	water	1 week	1 month	4 months	+4–25°C; protect from moisture and light	Bayer
Moxifloxacin (hydrochloride)	water	water	–	–	–	+4°C; protect from light and moisture	Bayer
Mupirocin (lithium)	water	water	–	–	–	+4°C; protect from light and moisture	GlaxoSmithKline
Nalidixic acid	water	water	–	–	–	+4°C; protect from light and moisture	Sanofi Winthrop
Netilmicin (sulphate)	water	water	6 months	6 months	6 months	+4°C; protect from light and moisture	Schering Plough
Nitrofurantoin	DMF	DMF	–	–	–	+4°C; protect from light and moisture	Proctor & Gamble
Norfloxacin	water	water	–	–	–	+4°C; protect from light and moisture	Merck Sharpe & Dohme Ltd
Ofloxacin	water	water	–	–	–	+4°C; protect from light and moisture	Aventis Pharma
Oxacillin (sodium)	water	water	–	–	–	+4°C; protect from light and moisture	GlaxoSmithKline
Penicillin (benzyl)[potassium]	water	water	–	1 month	1 month	+4°C; protect from light and moisture	GlaxoSmithKline
Piperacillin (sodium)	water	water	2 days	1 month	–	+4–25°C; protect from moisture and light	Wyeth Laboratories
Quinupristin/dalfopristin	water	water	–	1 month	–	2–8°C; protect from moisture and light	Aventis Pharma
Rifampicin (crystalline)	DMSO	water	1 month	1 month	–	+4°C; protect from light and moisture	Aventis Pharma
Roxithromycin	water	water	–	–	–	+4°C; protect from light and moisture	Aventis Pharma
Sparfloxacin	water	water	–	–	–	+4°C; protect from light and moisture	Aventis Pharma
Spectinomycin	water	water	–	–	–	+4°C; protect from light and moisture	Pharmacia & Upjohn Ltd
(dihydrochloride pentahydrate)	water	water	–	–	–	+4°C; protect from light and moisture	Medeva Pharma Ltd
Streptomycin (sulphate)	water	water	1 month	6 months	2 years	+4–25°C; protect from moisture and light	GlaxoSmithKline
Sulphamethoxazole (free acid)	water	water	–	–	–	+4°C; protect from light and moisture	Wyeth Laboratories
Tazobactam (sodium salt)	water	water	–	–	–	+4°C; protect from light and moisture	Aventis Pharma
Teicoplanin	water	water	–	NR <sup>i</sup>	NR <sup>i</sup>	unopened vials 2 years at RT	Wyeth Laboratories
Tetracycline (hydrochloride)	water	water	1 week	1 month	–	+4°C; protect from light and moisture	GlaxoSmithKline
Ticarcillin (sodium)	water	water	1 week	3 months	–	+4°C; protect from light and moisture	Eli Lilly & Co Ltd
Tobramycin (sulphate)	water	water	1 month	6 months	2 years	+4–25°C; protect from moisture and light	GlaxoSmithKline
Trimethoprim (base)	water	water	1 week	3 months	–	+4°C; protect from light and moisture	Eli Lilly & Co Ltd
Vancomycin (hydrochloride)	water	water	1 week	3 months	–	+4°C; protect from light and moisture	Eli Lilly & Co Ltd

<sup>a</sup>Many agents are available from Sigma, Poole, UK.

<sup>b</sup>Saturated NaHCO<sub>3</sub> solution.

<sup>c</sup>Ethanol.

<sup>d</sup>Phosphate buffer (0.1 M, pH 6).

<sup>e</sup>Water and 0.1 M NaOH dropwise to dissolve.

<sup>f</sup>Water (1 mL) + 10 μL glacial acetic acid.

<sup>g</sup>Phosphate buffer (0.07 M, pH 8).

<sup>h</sup>1 M MOPS pH 6.8 buffer.

<sup>i</sup>Precipitation 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 <sup>o</sup>	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

Determination of MICs

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.

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.