

Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents

European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)

FOREWORD

The purpose of this document is to promote the uniform application of terminology to methods used for the determination of the susceptibility of bacteria to antimicrobial agents, and to facilitate communication between the laboratory and the clinician, and between laboratories, nationally and internationally.

Definition of terms does not imply approval of any associated method or technique.

1 ANTIBIOTIC

An antibiotic is a substance of biological, semisynthetic or synthetic origin (the last strictly an antibacterial chemotherapeutic agent) that shows selective activity against bacteria, and is thus of potential use in the treatment of infection.

Disinfectants, antiseptics and preservatives are not included.

2 TEST SUBSTANCES

These are representatives of a group of antibiotics that are especially suitable for in vitro investigations, and which represent, optimally, the spectrum of activity of the group, and allow a minimum number of substances to be tested.

Ideally, in almost all cases, the antibiotic to be used clinically should be tested. This is possible only if the clinicians served by the laboratory have a common policy of restricted use, but results for other antibiotics may be useful in identification of resistance mechanisms (interpretive reading) and in identifying epidemiologic trends.

3 PATHOGEN CHARACTERISTICS (FIGURE 1)

3.1 Susceptible (S) (*also sensitive*)

This term is used in two senses, one microbiological and the other clinical. We have been unable to agree on terminology to resolve the resulting confusion. In each case, the definition is based on an analysis of the distribution of the results of measurements of one or other of the parameters used to define susceptibility, such as MIC (see section 6).

3.1.1 Microbiological susceptibility

Susceptible (sensitive) bacteria are those that belong to the most susceptible subpopulation and lack mechanisms of resistance, sometimes called the basic (susceptible) population.

3.1.2 Clinical susceptibility

Susceptible (sensitive) bacteria are those, defined on the basis of in vitro parameters, which ideally have been shown to respond to a standardized therapeutic regimen when causing infection. Some authorities distinguish **fully susceptible** bacteria (microbiologically susceptible) from **borderline** or **moderately susceptible** bacteria which, although they have a low-level resistance mechanism, usually respond to standard therapeutic regimens. The many other factors that affect response to therapy often make it difficult to determine the clinical effect of chemotherapy alone.

In the absence of reliable clinical information, the definition is based on a consensus interpretation of the antibiotic's in vitro properties and pharmacokinetics, and particularly on concentrations of antibiotic attainable at the site of infection (or often, as a practical approximation, in the blood).

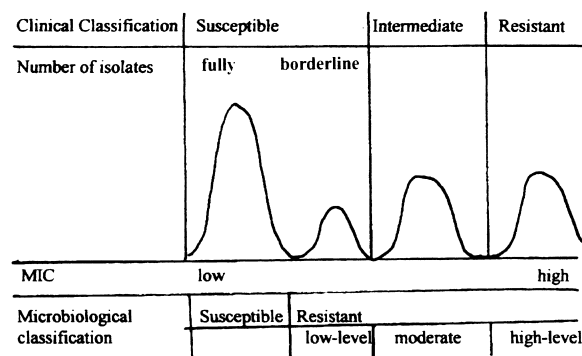


Figure 1 Hypothetical distribution of MICs among strains of bacteria isolated from infected patients, classified as clinically susceptible (fully or borderline), intermediate or resistant, and microbiologically susceptible or low-level, moderately or high-level resistant.

Bacteria with low-level microbiological resistance mechanisms may be clinically susceptible, though they are sometimes referred to as borderline susceptible.

3.2 Resistant

This term is used in two senses, one microbiological and the other clinical.

3.2.1 Microbiological resistance

Microbiologically **resistant** organisms are those that possess any resistance mechanism demonstrated either phenotypically or genotypically. The term may be qualified, as in 'moderately or highly resistant' or 'low-level or high-level resistance'. High-level resistance is associated with a lack of synergy between β -lactam antibiotics and aminoglycosides for enterococci.

Dissociated resistance can be demonstrated phenotypically only in the presence of a second antibiotic, as in macrolide resistance demonstrated in the presence of a lincosamine.

3.2.2 Clinical resistance

Clinical resistance occurs when infection is highly unlikely to respond even to maximum doses of a given antibiotic.

The many other factors that affect response to therapy often make it difficult to determine the clinical effect of chemotherapy alone, even when pathogens are resistant in vitro.

3.3 Intermediate

A bacterium is classified as being of **intermediate** susceptibility if it belongs to the group of strains that lies between the clinically susceptible and the clinically resistant. Infections caused by such strains have variable (or indeterminate) responses to chemotherapy, but may be eliminated if the antibiotic is concentrated at the site of the infection or the dosage is increased.

3.4 Cross-resistance

This is complete or partial insusceptibility to a group of antibiotics.

In its strict sense, cross-resistance applies to antibiotics of the same chemical class (e.g. β -lactams, aminoglycosides, or macrolides). However, resistance mechanisms such as those resulting from impermeability or efflux may affect more than one chemical class: this is sometimes referred to as **associated resistance**.

3.5 Susceptibility/resistance profile and antibiogram

These describe the pattern of susceptibility to a series of antibiotics.

A bacterium resistant to penicillin, streptomycin and tetracycline but susceptible to erythromycin may be described as having the **resistance profile** PST. Alternatively, such an organism may be described by the **antibiogram** RRRS for the predefined sequence of antibiotics.

3.6 Interpretive reading

Interpretive reading of susceptibility data is the deduction, from the observed phenotype, of biochemical mechanisms of resistance or the inference of clinical susceptibility or resistance.

4 SPECTRUM

The term **spectrum** characterizes the range of activity of an antibiotic agent against various bacterial species, or groups of species (e.g. Gram-positive, Gram-negative, aerobic, facultatively anaerobic, obligate anaerobes).

Acquired resistance may alter, from time to time and place to place, the pattern of susceptibility that originally defined the spectrum.

5 BREAKPOINTS

These are specific values of parameters such as MICs or inhibition zone diameters (which can be correlated with MICs by suitable statistical methods) on the basis of which bacteria can be assigned to the clinical categories 'susceptible (sensitive)', 'intermediate' and 'resistant'. The designation 'intermediate' also provides a technical buffer that minimizes confusion for organisms with MICs close to the breakpoint.

It is important to take particular note of the symbols used to define breakpoints: < means 'less than', while $\leq$ means 'equal to or less than', and > means 'greater than', while $\geq$ means 'equal to or greater than'.

6 ANTIBACTERIAL ACTION

6.1 Minimum inhibitory concentration (MIC)

This is the lowest concentration, expressed in mg/L (numerically equal to $\mu\text{g/mL}$, but we do not recommend the use of such units), that, under defined in vitro conditions, prevents the growth of bacteria within a defined period of time.

6.2 Minimum bactericidal concentration (MBC)

This is the lowest concentration of an antibiotic, expressed in mg/L, that under defined in vitro conditions reduces by 99.9% (3 logarithms) the number of organisms in a medium containing a defined inoculum of bacteria, within a defined period of time.

The reduction is usually expressed as the proportion of the inoculum (number of living CFUs introduced) that is rendered incapable of reproduction on subculture within that period.

The effects can be presented as a time-kill curve, in which an inoculum is incubated with the antibiotic and samples are tested for numbers of surviving CFUs at defined time intervals.

There are other definitions of MBC, and it is thus important to indicate which definition is being used.

6.3 The optimum bactericidal concentration (OBC) or most bactericidal concentration

This is the concentration of antibiotic that results in the maximum proportionate kill within a given time.

6.4 The minimum serum inhibitory concentration (titer)

This is the reciprocal of the highest dilution (titer) of a serum sample from a patient or volunteer (dosed with an antibiotic) that prevents an increase in the number of organisms capable of reproduction within a defined period of time. It is usually expressed as the titer that prevents an increase in turbidity of a liquid culture on incubation.

6.5 The minimum serum bactericidal concentration (titer)

This is the reciprocal of the highest dilution (titer) of a serum sample from a patient or volunteer that is able to reduce the number of organisms capable of reproduction as CFUs, usually by 99.9% (3 logarithms), within a defined period of time.

6.6 The minimum antibacterial concentration (MAC)

This is the concentration of an antibiotic, below the MIC as defined above, that can exert specified biological effects on bacteria.

Such effects may include partial inhibition of growth below the MIC, changes in bacterial morphology, changes in adhesion to surfaces, acceleration of phagocytosis, increase or decrease of antimicrobial activity in combination with other antibiotics, prolongation of generation times or changes in toxin production.

6.7 Tolerance

Tolerance occurs when, under defined conditions, a test substance that is usually bactericidal for the bacteria tested shows a diminished or absent bactericidal effect without loss of inhibitory action (no change occurs in the MIC). The difference between MIC and MBC is usually quantified (e.g. 16–32-fold). Tolerance renders a normally bactericidal agent bacteriostatic.

6.8 Persistence

Persistence occurs when individual cells (persisters) survive the effects of an antibiotic, despite its concentration being above the MBC for the population as a whole.

On retesting, persisters demonstrate the same susceptibility as the original population as a whole and no larger proportion of cells persists.

6.9 Paradoxical effect (Eagle phenomenon)

This occurs when, in tests to determine the MBC of an antibiotic, organisms survive in significantly larger numbers on subculture from media containing antibiotic concentrations above, but not immediately above, the MBC.

6.10 Postantibiotic effect (PAE)

This is the delay of growth of bacteria (compared with that of a suitable control population containing the same number of bacteria) after a limited period of exposure to an antibiotic, after which the effect of the antibiotic is removed (e.g. by ≥ 100 -fold dilution or by filtration and washing). The term may also be applied to morphologic or biochemical changes. **Adaptive resistance** is a reduction in susceptibility within the basic range, without a demonstrable genetic determinant.

6.11 Postantibiotic effect index (PAE index)

This is the time needed for the cell count (CFU/mL) of a population of microorganisms exposed to the MIC or more of an antibiotic over a defined period to increase by a factor of 10 (1 log) after removal or dilution of the antibiotic, in comparison with an untreated (but suitably diluted) control culture.

7 PROPERTIES OF ANTIBIOTICS

7.1 Potency

This is the amount of antibacterially active agent in a test substance, determined by means of a bioassay, usually expressed in micrograms per milligram ($\mu\text{g}/\text{mg}$) of test substance.

7.2 Concentration

This is the amount of an antimicrobial agent in a defined volume of liquid, preferably expressed as mg/L (rather than $\mu\text{g/mL}$ or mcg/mL), or in a defined mass of a solid, usually expressed as $\mu\text{g/g}$ or mg/kg .

7.3 Pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of drug concentrations over time, in different body compartments, after a given dose of an antibiotic.

Pharmacodynamics is the study of the relationship between pharmacokinetic parameters and the magnitude and time course of the response of the pathogen.

7.4 MIC₅₀, MIC₉₀, etc.

This is the concentration of antibiotic that inhibits 50%, 90%, etc. of the isolates or strains in a collection.

8 EFFECT OF COMBINATIONS OF ANTIBIOTICS (TABLE 1)

The effects described occur *in vitro*: their clinical significance is unclear.

8.1 Indifference

An **indifferent** effect of a combination of antibiotics or antibiotics and inactive substances is one that is equal to the effects of the most active component.

If one substance is more active than another, indifference is difficult to differentiate from an additive effect, especially when doubling dilutions of antibiotic are used.

8.2 Additive effects

The **additive** effect of a combination of antibiotics is one in which the effect of the combination is equal to that of the sum of the effects of the individual components.

8.3 Synergism

Synergistic action of a combination of antibiotics or antibiotics and inactive substances is present if the effect of the combination exceeds the additive effects of the individual components.

8.4 Antagonism

Antagonism is present if a reduced effect of a combination of antibiotics is observed in comparison with the effect of the most effective individual substance.

8.5 An isobologram

This is the figurative representation of the interaction of two antibiotics (Figure 2).

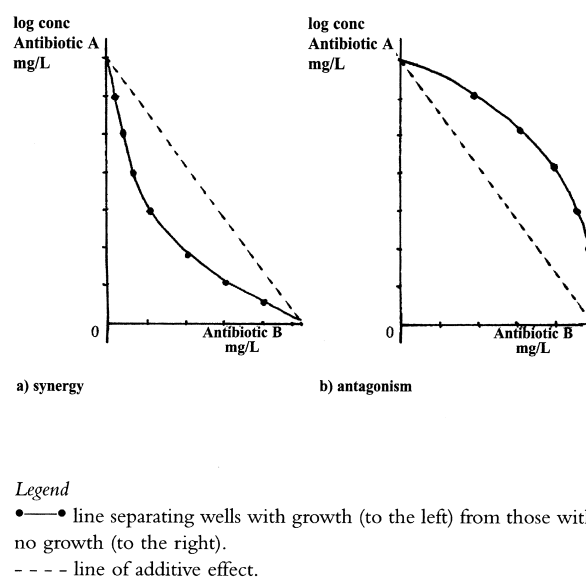


Figure 2 Interpretation of an isobologram for two antibiotics A and B in a checkerboard titration.

Table 1 Correlation between FIC and FBC and the effect of the combination of antibacterial agents

Index	Synergy	Additive	Indifference	Antagonism
FIC or FBC	≤ 0.5	$> 0.5-1$	> 1 to < 2	≥ 2

Note: This does not take account of the error introduced by the use of doubling solutions.

8.6 Fractional inhibitory concentration (FIC), fractional bactericidal concentration (FBC), FIC index and average FIC index

The **fractional inhibitory concentration** and the **fractional bactericidal concentration** are mathematical expressions of the effect of the combination of antibacterial agents (see sections 8.1–8.4).

For two antibiotics A and B acting alone and in combination:

$$FIC_{(A)} = \frac{MIC_{(A \text{ in the presence of B})}}{MIC_{(A \text{ alone})}}$$

$$FIC_{(B)} = \frac{MIC_{(B \text{ in the presence of A})}}{MIC_{(B \text{ alone})}}$$

The ΣFIC (FIC index) may be calculated according to the following equation:

$$\Sigma FIC = FIC_{(A)} + FIC_{(B)}$$

Checkerboard titration assays result in a number of FIC indices. The sum of a number of FIC indices divided by the number of indices is designated as average ΣFIC . The synergism/antagonism criteria given in Table 1 apply.

The FBC may be calculated in the same way by replacing the MIC values with the MBC values. The evaluation of the FIC and FBC is summarized in Table 1.

8.7 Time-kill curves (Figure 3)

These may also be used to present data on interactions.

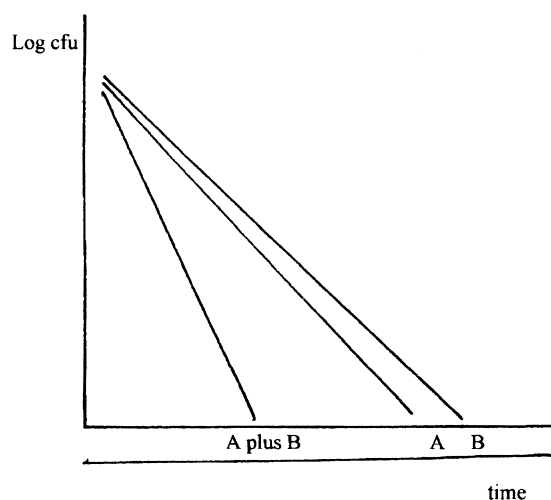


Figure 3 Hypothetical time-kill curves for two antibiotics A and B acting on a bacterial culture alone and in combination demonstrating synergism (the difference in $\Delta \log CFU/h$ for the combination being ≥ 2 -fold that of the most active component).

9 STOCK SOLUTION

A **stock solution** of an antibiotic is the starting solution used for further dilution.

10 REFERENCE STRAINS

Reference strains are catalogued, characterized bacteria with stable, defined antibiotic susceptibility phenotypes.

Reference strains are kept as stock cultures, from which are derived working cultures. They are obtainable from culture collections and used for quality control.

11 SUSCEPTIBILITY (SENSITIVITY) TESTING METHODS

11.1 Broth dilution

Broth dilution is a technique in which containers are filled with identical volumes of inoculated broth and identical volumes of an antibiotic solution, but incrementally (usually geometrically) increasing concentrations of the antibiotic, and a defined inoculum. The aim of this method is the determination of the lowest concentration that inhibits bacterial growth—the **minimum inhibitory concentration (MIC)** or other parameters (see sections 11.3 and 11.4). The broth dilution method may be performed by macrodilution or microdilution.

11.1.1 Macrodilution

This denotes the performance of the broth dilution method in tubes containing a minimum volume of 2 mL.

11.1.2 Microdilution

This denotes the performance of the broth dilution method in microtiter plates with a capacity of $\leq 500 \mu L$ per well.

11.2 Agar dilution

Agar dilution involves the incorporation of an antimicrobial agent in solid or semisolid agar media in a geometric progression of concentrations and the application of a defined bacterial inoculum to the surface. Its purpose is the determination of the lowest concentration that inhibits bacterial growth—**minimum inhibitory concentration (MIC)**. (See also sections 12, 13, 14 and 15.)

11.3 MBC determination

Containers are filled with identical volumes of inoculated broth and identical volumes of an antibiotic, but incrementally (geometrically) increasing concentrations of the antibiotic, and a defined bacterial inoculum. Its purpose is the determination of the lowest concentration that reduces the number of viable bacteria present by more than 99.9% after a defined exposure time and subculture to antibiotic-free medium—the **minimum bactericidal concentration (MBC)**.

11.4 Killing kinetics

Time-kill curves are figurative representations (Figure 3) of bacterial concentrations (CFU/mL) in subcultures taken serially from cultures, usually in liquid media, containing antibiotic(s). Killing kinetics, including the speed of kill ($\Delta \log$ CFU/h), can be derived from them.

11.5 Agar diffusion

Diffusion of the antibiotic from disks, tablets or strips in solid inoculated culture media gives rise to inhibition zones whose diameters correlate with the minimum inhibitory concentrations (MICs) for the bacteria.

12 MEDIUM

The **medium** is a culture medium (a preparation used for the in vitro growth of bacteria), which may be solidified with agar (a complex polysaccharide derived from seaweeds), which is used for the agar or broth dilution methods and the agar diffusion test.

13 INOCULUM

The **inoculum size** is the number of bacteria in a suspension, calculated with respect to the final volume. It is expressed as colony-forming units per milliliter (CFU/mL), although it may be defined less accurately in terms of the turbidity or optical density of the suspension. An **inoculum effect** is a change in susceptibility related to change in inoculum size.

14 INHIBITION ZONE

The **inhibition zone** is the area free of growth in a bacterial lawn which results from the inhibitory effect of antibiotic that has diffused into the medium from its applied source.

14.1 Inhibition zone diameter

This is measured in millimeters.

14.2 Inhibition zone 'radius'

This is the distance in millimeters from the edge of an antibiotic disk to the edge of the zone of inhibition of growth (i.e. the radius of the zone of inhibition minus the radius of the disk).

15 VEHICLES

Vehicles serve as carriers for the antimicrobial agent for the determination of the susceptibility of a bacterial strain to be

tested to the respective antimicrobial agents by means of the diffusion or dilution tests. Vehicles may be paper disks, strips or tablets loaded with defined amounts of antibiotics, and labeled accordingly. In the past, other vehicles, such as wells or cylinders, were used.

15.1 Batch or lot

A **batch** or **lot** comprises all vehicles consisting of the same material and produced from the same starting solution of an antimicrobial agent in an uninterrupted process.

15.2 Disk content

The **disk content** of antibiotic is defined in micrograms or units, and never as a concentration. The **tolerance** is the range of antibiotic load permitted to the manufacturer around the stated amount in a vehicle (usually 90–120%); this has no relationship with tolerance as defined in section 6.7 above.

15.3 Standardized abbreviations

Standardized abbreviations of antibiotic names, as used on susceptibility testing disks, have not yet been agreed by EUCAST.

15.4 Expiry date

An expiry date is given for each lot of loaded vehicles. It indicates that, up to the specified date, no fall in activity to $\leq 90\%$ of the declared activity occurs under defined storage conditions. The expiry date is determined following stability testing.

16 CULTURE CONDITIONS

Culture is the intentional growth of bacteria in a controlled environment.

During **incubation**, cultures are held at a suitable temperature (normally 35–37 °C) for a suitable time (normally overnight) in a suitable gaseous atmosphere.

REVISIONS

This document was approved by EUCAST in May 2000.

Revisions will be considered by the Terminology Subcommittee of EUCAST in January 2001. Proposals for changes should be sent to EUCAST via Cornelia Hasselmann (Tel: + 49 89 89712003; Fax: + 49 89 89712004).