INTERNATIONAL STANDARD

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Clinical laboratory testing and *in vitro* diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

Systèmes d'essais en laboratoire et de diagnostic in vitro — Essais de réceptivité d'agents infectieux et évaluation des performances des dispositifs de réceptivité antimicrobienne —

Partie 1: Méthode de référence pour la détermination de la sensibilité in vitro aux agents microbiens des bactéries aérobies à croissance rapide impliquées dans les maladies infectieuses

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 20776-1 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, In vitro *diagnostic medical devices*, in collaboration with Technical Committee ISO/TC 212, *Clinical laboratory testing and* in vitro *diagnostic test systems*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

ISO 20776 consists of the following parts, under the general title *Clinical laboratory testing and* in vitro *diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices*:

— *Part 1: Reference method for testing the* in vitro *activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases*

— *Part 2: Evaluation of performance of antimicrobial susceptibility test devices*

Introduction

In vitro susceptibility tests are performed on microorganisms suspected of causing disease, particularly if the organism is thought to belong to a species that may exhibit resistance to frequently used antimicrobial agents. The tests are also important in resistance surveillance, epidemiological studies of susceptibility and in comparisons of new and existing agents.

Dilution procedures are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference method for antimicrobial susceptibility testing. MIC methods are used in resistance surveillance, comparative testing of new agents, to establish the susceptibility of organisms that give equivocal results in routine tests, for tests on organisms where routine tests may be unreliable and when a quantitative result is required for clinical management. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in broth (broth dilution) containing serial dilutions of the antimicrobial agent.

The lowest concentration of an antimicrobial agent (in mg/l) that, under defined *in vitro* conditions, prevents the appearance of visible growth of a microorganism within a defined period of time is known as the MIC. The MIC is a guide for the clinician to the susceptibility of the organism to the antimicrobial agent and aids treatment decisions. Careful control and standardisation is required for intra- and inter-laboratory reproducibility, as results may be significantly influenced by the method used. It is generally accepted that broth MIC tests are reproducible to within one doubling dilution of the real end point (i.e. ± one well or tube in a doubling dilution series).

Broth dilution is a technique in which containers holding identical volumes of broth with antimicrobial agent solutions in incrementally (usually geometrically) increasing concentrations are inoculated with a known number of microorganisms.

Broth microdilution denotes the performance of the broth dilution test in microdilution trays.

The method described in this part of ISO 20776 is intended for the testing of pure cultures of aerobic bacteria that are easily grown by overnight incubation on agar and grow well in Mueller-Hinton broth, which may be supplemented. The broth microdilution method described in this part of ISO 20776 is essentially the same as those used in many countries, including France[1], Germany[2], Sweden[3], the United Kingdom[4], and the United States[5]. The method is also essentially the same as the broth microdilution method published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[6]. All these methods are based on those described by Ericsson and Sherris[7].

Clinical laboratory testing and *in vitro* diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

WARNING — The use of this part of ISO 20776 may involve hazardous materials, operations and equipment. This part of ISO 20776 does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this part of ISO 20776 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 20776 describes one reference method, broth microdilution, for determination of MICs. The MIC reflects the activity of the drug under the described test conditions, and can be interpreted for clinical management purposes by taking into account other factors, such as drug pharmacology or bacterial resistance mechanisms. This allows categorization of bacteria as “susceptible” (S), “intermediate” (I), or “resistant” (R). In addition, MIC distributions can be used to define wild type or non-wild type bacterial populations. Although clinical interpretation of the MIC value is beyond the scope of this part of ISO 20776, modifications of the basic method are required for certain antimicrobial agent - bacteria combinations to facilitate clinical interpretation. These modifications are included in a separate table. It is advisable to compare other susceptibility testing methods (e.g. routine methods or diagnostic test devices) with this reference method for validation, in order to ensure comparable and reliable results.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
antimicrobial agent
substance of biological, semi-synthetic or synthetic origin that inhibits the growth of or kills bacteria, and is thus of potential use in the treatment of infections

NOTE Disinfectants, antiseptics and preservatives are not included in this definition.

2.2 Antimicrobial agents — properties

2.2.1
potency
antimicrobially active fraction of a test substance, determined in a bioassay against a reference powder of the same substance

NOTE The potency is expressed as mass fraction in milligrams per gram (mg/g), or as activity content in International Units (IU) per gram, or as a volume fraction or mass fraction in percent, or as an amount-of-substance concentration (mass fraction) in mole per litre of ingredients in the test substance.

2.2.2
concentration
amount of an antimicrobial agent in a defined volume of liquid

NOTE 1 The concentration is expressed as mg/l.

NOTE 2 mg/l ≡ µg/ml but it is not recommended to use the unit µg/ml.

2.3
stock solution
initial solution used for further dilutions

2.4
minimum inhibitory concentration
MIC
lowest concentration that, under defined *in vitro* conditions, prevents visible growth of bacteria within a defined period of time

NOTE The MIC is expressed in mg/l.

2.5
breakpoint
BP
specific values of parameters, such as MICs, on the basis of which bacteria can be assigned to the clinical categories “susceptible”, “intermediate” and “resistant”

NOTE For current interpretive breakpoints, reference can be made to the latest publications of organizations employing this reference method (e.g. CLSI and EUCAST).

2.5.1
susceptible
S
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success

NOTE 1 Bacterial strains are categorized as susceptible by applying the appropriate breakpoints in a defined phenotypic test system.

NOTE 2 This breakpoint can be altered due to changes in circumstances (e.g. changes in commonly used drug dosages, emergence of new resistance mechanisms).

2.5.2
intermediate
I
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with uncertain therapeutic effect

NOTE 1 Bacterial strains are categorized as intermediate by applying the appropriate breakpoints in a defined phenotypic test system.

NOTE 2 This class of susceptibility implies that an infection due to the isolate can be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used.

NOTE 3 This class also indicates a “buffer zone”, to prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

NOTE 4 These breakpoints can be altered due to changes in circumstances (e.g. changes in commonly used drug dosages, emergence of new resistance mechanisms).

2.5.3
resistant
R
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure

NOTE 1 Bacterial strains are categorized as resistant by applying the appropriate breakpoints in a defined phenotypic test system.

NOTE 2 This breakpoint can be altered due to changes in circumstances (e.g. changes in commonly used drug dosages, emergence of new resistance mechanisms).

2.6
wild type
absence of acquired resistance mechanisms to the antimicrobial agent for a given strain

2.7
reference strain
catalogued, characterized bacteria with stable, defined antimicrobial susceptibility phenotypes and/or genotypes

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

2.8 Susceptibility testing method

2.8.1
broth dilution
technique in which containers are filled with appropriate volumes of an antimicrobial solution, employing incrementally (usually two-fold) increasing concentrations of the antimicrobial agent and appropriate volumes of broth with a defined inoculum

NOTE The aim of this method is the determination of the MIC.

2.8.2
microdilution
performance of broth dilution in microdilution trays with a capacity of $\leqslant$ 200 µl per well

2.9
broth
fluid medium used for the *in vitro* growth of bacteria

2.10
inoculum
number of bacteria in a suspension, calculated with respect to the final volume

NOTE The inoculum is expressed as colony-forming units per millilitre (CFU/ml).

2.11
inoculum effect
change in MIC related to change in inoculum

3 Test procedures

3.1 General

The tests are performed in microdilution trays. The method is based on the preparation of antimicrobial agent working solutions, either in 50 µl volumes per well (with the addition of an inoculum also in a volume of 50 µl), or in a volume of 100 µl per well (with the addition of a maximum of 5 µl inoculum volume).

3.2 Medium

Mueller-Hinton broth shall be used (see Annex A for details).

3.3 Antimicrobial agents

3.3.1 General

Antimicrobial agents shall be obtained directly from the manufacturer or from reliable commercial sources; pharmaceutical preparations for clinical use are not acceptable. The antimicrobial agents shall be supplied with a lot number, potency, an expiry date and details of recommended storage conditions. Substances shall be stored in tightly closed containers in the dark, at 4 °C to 8 °C, with a desiccant unless otherwise recommended by the manufacturer. Hygroscopic agents should be dispensed into aliquots, one of which is used on each test occasion.

Allow containers to warm to room temperature before opening them to avoid condensation.

3.3.2 Preparation of stock solutions

The use of a calibrated analytical balance is required to weigh antimicrobial agents. Allowance for the potency of the powder shall be made by use of the following formula to obtain the amount of antimicrobial agent substance or the volume of diluent needed for a standard solution:

$$m = \frac{V \times \rho}{P} \qquad (1)$$

$$V = \frac{m \times P}{\rho} \qquad (2)$$

where

ρ is the concentration of the stock solution, in mg/l;

m is mass of the antimicrobial agent (powder), in g;

P is the potency of the antimicrobial agent (powder), in mg/g;

V is the volume of diluent, in l.

Concentrations of stock solutions should be 1 000 mg/l or greater, although the solubility of some agents is a limiting factor. The actual concentrations of stock solutions depend on the method of preparing working solutions (serial dilutions). Agents should be dissolved and diluted in sterile distilled water unless the manufacturer states otherwise. Some agents require alternative solvents (see Table 1). Sterilisation of solutions is not usually necessary. If required, sterilisation should be done by membrane filtration, and samples before and after sterilisation should be compared by assay to ensure that adsorption has not occurred.

Unless information is available on stability of stock solutions under specified storage conditions, they should be prepared fresh for each test batch.

