SLOVENSKI STANDARD
oSIST prEN ISO 20776-1:2018

01-september-2018

Preskus občutljivosti povzročiteljev infekcij na delovanje antimikrobno občutljivih naprav - 1. del: Referenčna metoda za preskus aktivnosti in vitro antimikrobnih povzročiteljev na vpliv bakterij pri nalezljivih boleznih (ISO/DIS 20776-1:2018)

Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases (ISO/DIS 20776-1:2018)

Labormedizinische Untersuchungen und In-vitro-Diagnostika-Systeme - Empfindlichkeitsprüfung von Infektionserregern und Evaluation von Geräten zur antimikrobiellen Empfindlichkeitsprüfung - Teil 1: Referenzmethode zur Testung der In-vitro-Aktivität von antimikrobiellen Substanzen gegen schnell wachsende aerobe Bakterien, die Infektionskrankheiten verursachen (ISO/DIS 20776-1:2018)

Sensibilité in vitro des agents infectieux et évaluation des performances des dispositifs pour antibiogrammes - Partie 1: Méthode de référence pour la détermination de la sensibilité in vitro aux agents antimicrobiens des bactéries aérobies à croissance rapide impliquées dans les maladies infectieuses (ISO/DIS 20776-1:2018)

Ta slovenski standard je istoveten z: **prEN ISO 20776-1**

ICS:

11.100.10	Diagnostični preskusni sistemi in vitro	In vitro diagnostic test systems

oSIST prEN ISO 20776-1:2018 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 20776-1

ISO/TC **212**

Secretariat: **ANSI**

Voting begins on:
2018-06-26

Voting terminates on:
2018-09-18

Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 1:
Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

Sensibilité in vitro des agents infectieux et évaluation des performances des dispositifs pour antibiogrammes —

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

ICS: 11.100.20

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 20776-1:2018(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

This second edition cancels and replaces the first edition (ISO 20776-1:2006), which has been technically revised.

The main changes compared to the previous edition are as follows:

— Revised to a broth micro-dilution only performance document

— Removal of S,I, R breakpoint definitions and information

— Moved embedded Tables to Annexes

— Removed quality control range Table.

— Updated Table (now Annex B) on solvents and diluents for antimicrobial agents used globally.

— Updated information on special culture media and method performance for specific currently used antimicrobial agents.

A list of all parts in the ISO 20776 series can be found on the ISO website.

Introduction

In vitro antimicrobial susceptibility tests are performed on micro-organisms 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 for antimicrobial susceptibility testing. MIC methods are used in resistance surveillance, defining identifying wild type phenotypes, 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, micro-organisms are tested for their ability to produce visible growth in broth (broth dilution) containing serial dilutions of the antimicrobial agent or on a series of agar plates (agar dilution).

The lowest concentration of an antimicrobial agent (in mg/l) that, under defined in vitro conditions, prevents the appearance of visible growth of a micro-organism 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 standardizationis required for intra- and inter-laboratory reproducibility of broth MIC tests. The MICs generally span two to three doubling dilutions with a dominant central value.

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 micro-organisms.

Broth micro-dilution denotes the performance of the broth dilution test in micro-dilution 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 standardised micro-dilution trays containing standardised Mueller-Hinton broth (volume of ≤ 200 µl), which may need to be modified depending on the antimicrobial agent being tested.

The broth micro-dilution method described in this part of ISO 20766 is essentially the same as those used in many countries, and as the methods published by the Clinical and Laboratory Standards Institute (CLSI)[1] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[2]. These methods are based on those described by Ericsson and Sherris[3].

Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: Broth micro-dilution 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 micro-dilution, for determination of MICs. The MIC may be a guide for the clinician, and reflects the activity of the drug under the described test conditions, by taking into account other factors, such as drug pharmacology, pharmacokinetics, or bacterial resistance mechanisms. This allows categorisation 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 Annex of this document. It is necessary to compare other susceptibility testing methods (e.g. disc diffusion or diagnostic test devices) with this reference method for validation, in order to ensure comparable and reliable results.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
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 to entry: Disinfectants, antiseptics and preservatives are not included in this definition.

3.2 Antimicrobial agents — properties

3.2.1
potency
potency is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity.

Note to entry: 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 (molar fraction) in mole per litre of ingredients in the test substance.

3.2.2
concentration
amount of an antimicrobial agent in a defined volume of liquid

Note 1 to entry: The concentration is expressed as mg/l.

Note 2 to entry: mg/l is the designated ISO unit.

3.3
stock solution
initial solution used for further dilutions

3.4
minimum inhibitory concentration
MIC
lowest concentration that, under defined *in vitro* conditions, prevents visible growth of bacteria within a defined period of time

Note to entry: The MIC is expressed in mg/l.

3.5
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 to entry: For current interpretive breakpoints, reference can be made to the latest publications of organisations employing this reference method (e.g. CLSI and EUCAST).

3.6
wild type
absence of known resistance mechanisms to the antimicrobial agent for a given strain

3.7
reference strain
catalogued, characterized bacteria with stable, defined antimicrobial susceptibility phenotypes and/or genotypes

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

3.8 Susceptibility testing method

3.8.1
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 to entry: The aim of this method is the determination of the MIC.

3.8.2
micro-dilution
performance of broth dilution in micro-dilution trays with a final test volume of ≤ 200 µl per well

3.9
broth
fluid medium used for the *in vitro* growth of bacteria

Note to entry: For the broth reference method the medium is standardised Mueller-Hinton broth (see Annex A)

3.10
inoculum
number of bacteria in a suspension, calculated with respect to the final volume

Note to entry: The inoculum is expressed as colony-forming units per millilitre (CFU/ml).

3.11
inoculum effect
change in MIC related to change in inoculum concentration

4 Test procedures

4.1 General

The tests are performed in polystyrene micro-dilution 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 10 µl inoculum volume).

4.2 Medium

Mueller-Hinton broth shall be used (see Annex A for details and Annex D for special test situations).

4.3 Antimicrobial agents

4.3.1 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 as powders 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, with a desiccant at the recommended temperature of the supplier. Hygroscopic agents should be dispensed into aliquots, one of which is used on each test occasion.