

DIN EN ISO 20776-1



ICS 11.100.20

Supersedes
DIN EN ISO 20776-1:2007-02

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 20776-1:2019, including Corrected version 2019-12);

English version EN ISO 20776-1:2020,

English translation of DIN EN ISO 20776-1:2020-12

Empfindlichkeitsprüfung von Infektionserregern und Leistungsbewertung 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 20776-1:2019, einschließlich der korrigierten Fassung von 2019-12);

Englische Fassung EN ISO 20776-1:2020,

Englische Übersetzung von DIN EN ISO 20776-1:2020-12

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

Partie 1: Méthode de référence de microdilution en bouillon pour la détermination de la sensibilité in vitro aux agents antimicrobiens des bactéries aérobies à croissance rapide impliquées dans les maladies infectieuses (ISO 20776-1:2019, y compris version corrigée 2019-12);

Version anglaise EN ISO 20776-1:2020,

Traduction anglaise de DIN EN ISO 20776-1:2020-12

Document comprises 26 pages

Translation by DIN-Sprachendienst.

In case of doubt, the German-language original shall be considered authoritative.

A comma is used as the decimal marker.

National foreword

This document (EN ISO 20776-1:2020) has been prepared by Technical Committee ISO/TC 212 “Clinical laboratory testing and in vitro diagnostic test systems” in collaboration with Technical Committee CEN/TC 140 “In-vitro-diagnostic medical devices” (Secretariat: DIN, Germany).

The responsible German body involved in its preparation was NA 063 BR *Beirat des DIN-Normenausschuss Medizin* (Board of DIN Standards Committee Medicine).

DIN EN ISO 20776 consists of the following parts, under the general title *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*
- Part 2: *Evaluation of performance of antimicrobial susceptibility test devices*

For current information on this document, please go to DIN’s website (www.din.de) and search for the document number in question.

Amendments

This standard differs from DIN EN ISO 20776-1:2007-02 as follows:

- a) the document has been revised to refer to the performance of broth micro-dilution only;
- b) S, I, R breakpoint definitions and information have been deleted;
- c) tables have been moved to the annexes;
- d) the table on quality control has been deleted;
- e) the table on solvents and diluents for antimicrobial agents used globally has been updated (now Annex B);
- f) information on special culture media and method performance for specific currently used antimicrobial agents has been updated;
- g) the standard has been editorially revised.

Previous editions

DIN EN ISO 20776-1: 2007-02

English Version

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 20776-1:2019, including Corrected version 2019-12)

Sensibilité in vitro des agents infectieux et évaluation des
performances des dispositifs pour antibiogrammes -
Partie 1: Méthode de référence de microdilution en
bouillon pour la détermination de la sensibilité in vitro
aux agents antimicrobiens des bactéries aérobies à
croissance rapide impliquées dans les maladies
infectieuses
(ISO 20776-1:2019, y compris version
corrigée 2019-12)

Empfindlichkeitsprüfung von Infektionserregern und
Leistungsbewertung 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 20776-1:2019, einschließlich der korrigierten
Fassung von 2019-12)

This European Standard was approved by CEN on 22 April 2020.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents	Page
European foreword	3
Foreword	4
Introduction	5
1 Scope	6
2 Normative references	6
3 Terms and definitions	6
4 Test procedures	8
4.1 General	8
4.2 Medium	8
4.3 Antimicrobial agents	8
4.3.1 General	8
4.3.2 Preparation of stock solutions	8
4.3.3 Preparation of working solutions	9
4.3.4 Preparation of micro-dilution trays	9
4.3.5 Storage of micro-dilution trays	9
4.4 Preparation of inoculum	10
4.4.1 General	10
4.4.2 Broth culture method	10
4.4.3 Direct colony suspension method	10
4.5 Inoculation of micro-dilution trays	10
4.6 Incubation of micro-dilution trays	11
4.7 Reading results	11
4.8 Special test situations where the MIC result might give unreliable results	11
5 Quality control	11
Annex A (informative) Requirements for Mueller-Hinton broth	13
Annex B (informative) Solvents and diluents for making stock solutions of selected antimicrobial agents	16
Annex C (informative) Preparation of working dilutions of antimicrobial agents for use in broth dilution susceptibility tests	21
Annex D (informative) Special test situations	22
Bibliography	23

European foreword

This document (EN ISO 20776-1:2020) has been prepared by Technical Committee ISO/TC 212 "Clinical laboratory testing and in vitro diagnostic test systems" in collaboration with Technical Committee CEN/TC 140 "In vitro diagnostic medical devices" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2021, and conflicting national standards shall be withdrawn at the latest by July 2023.

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

This document supersedes EN ISO 20776-1:2006.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 20776-1:2019, including Corrected version 2019-12 has been approved by CEN as EN ISO 20776-1:2020 without any modification.

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 of 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 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.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

This corrected version of ISO 20776-1:2019 incorporates the following correction:

- Correction of the diluent pH value for ampicillin from 8,0 to 6,0 in [Annex B](#).

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 standardization is 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 document is intended for the testing of pure cultures of aerobic bacteria that are easily grown by overnight incubation on agar and grow well in standardized micro-dilution trays containing standardized Mueller-Hinton broth (volume of $\leq 200 \mu\text{l}$), which may need to be modified depending on the antimicrobial agent being tested.

The broth micro-dilution method described in this document 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].

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

1 Scope

This document describes one reference method, broth micro-dilution, for determination of MICs. The MIC can 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 document, 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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

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

3.2 potency

measure of drug activity expressed in terms of the amount required to produce an effect of given intensity

Note 1 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.

3.3

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.

3.4

stock solution

initial solution used for further dilutions

3.5

minimum inhibitory concentration

MIC

lowest concentration that, under defined in vitro conditions, prevents visible growth of bacteria within a defined period of time

Note 1 to entry: The MIC is expressed in mg/l.

3.6

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 1 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).

3.7

wild type

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

3.8

reference strain

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

Note 1 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.

3.9

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

3.10

micro-dilution

performance of broth dilution in micro-dilution trays with a final test volume of $\leq 200 \mu\text{l}$ per well

3.11
broth

fluid medium used for the in vitro growth of bacteria

Note 1 to entry: For the broth reference method the medium is standardised Mueller-Hinton broth (see [Annex A](#)).

3.12
inoculum

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

Note 1 to entry: The inoculum is expressed as colony-forming units per millilitre (CFU/ml).

3.13
inoculum effect

change in MIC related to change in inoculum in CFU/ml

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.

To avoid condensation, allow containers to warm to room temperature before opening.

4.3.2 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:

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

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

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 [Annex B](#)).

NOTE For newer antimicrobial agents not identified in [Annex B](#) of this current document, consult manufacturer information on the most appropriate solvent and diluent for the specific agent. 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.

4.3.3 Preparation of working solutions

The range of concentrations selected for testing depends on the micro-organisms and antimicrobial agent. The chosen range shall allow full endpoint MIC determination for appropriate reference strains. A two-fold dilution series based on 1 mg/l is prepared in Mueller-Hinton broth. Dilutions should not be prepared by serial dilution steps, but according to the procedure outlined in [Annex C](#). Working solutions shall be used the same day unless information is available on stability of the solutions under specified storage conditions.

4.3.4 Preparation of micro-dilution trays

Working solutions are dispensed into micro-dilution trays at 50 μ l per well with double the desired final concentrations of antimicrobial agent, or at 100 μ l per well in the desired final concentrations.

At least one well, containing 50 μ l or 100 μ l of antimicrobial agent-free medium, should be included as a growth control for each strain tested. Likewise, a well containing 100 μ l of antimicrobial agent-free medium should be included as an un-inoculated negative control well for each micro-organism type tested.

4.3.5 Storage of micro-dilution trays

Filled trays may be used immediately or may be stored for up to three months or until documented quality control or other evidence indicates degradation of the antimicrobial agent. For storage the filled trays should be sealed in plastic bags and immediately placed in a freezer at ≤ -60 °C unless the antimicrobial agents are known to be stable at higher temperatures.

Trays shall not be stored in a self-defrosting freezer, and thawed antimicrobial solutions shall not be re-frozen, as repeated freeze-thaw cycles accelerate the degradation of some antimicrobial agents, particularly β -lactams.

Allow frozen plates to thaw for up to 2 h and inoculate by 4 h of removal from the freezer.

4.4 Preparation of inoculum

4.4.1 General

Standardization of the inoculum is essential for accurate and reproducible broth micro-dilution susceptibility tests. Therefore, purity checks and viable colony counts shall be performed on every isolate tested with this reference procedure

4.4.2 Broth culture method

The inoculum may be prepared by diluting a broth culture or by suspending several morphologically similar colonies (when possible) from an overnight culture on non-selective agar medium in broth with a sterile loop or swab. When suspending colonies, morphologically similar colonies should be picked to avoid contamination of other species or atypical variants of the same species.

The broth used shall not be antagonistic to the antimicrobial agent tested. The broth is incubated at $(35 \pm 1)^\circ\text{C}$ until the growth reaches a turbidity equal to or greater than that of a 0,5 McFarland standard^[4]. If needed, the culture is adjusted with saline or broth to give a turbidity equivalent to the 0,5 McFarland standard. This can be done by means of a photometric device (using 625 nm wavelength and a 1 cm path cuvette, the absorbance will be in the range of 0,08 to 0,13), or by employing a suitably calibrated nephelometer. Alternatively, this can be achieved visually by comparing the appearance of black lines through the inoculum and 0,5 McFarland standard suspensions (the inoculum and McFarland standard shall be in tubes of the same size) or any other method that gives reproducible CFU/ml. The final inoculum shall be 5×10^5 CFU/ml (target range, 2×10^5 CFU/ml to 8×10^5 CFU/ml).

NOTE A 0,5 McFarland standard can be produced by adding a 0,5 ml aliquot of 0,048 mol/l barium chloride (BaCl_2) (11,72 g/l $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) to 99,5 ml of 0,18 mol/l sulphuric acid (H_2SO_4), with constant stirring to maintain a suspension.

4.4.3 Direct colony suspension method

Several morphologically similar colonies from a non-selective nutritive agar medium, incubated at $35 \pm 1^\circ\text{C}$ for 18 h to 24 h, unless longer incubation is required, are touched with a sterile loop and the growth transferred to sterile broth or saline. The suspension is adjusted to give a turbidity equivalent to that of a 0,5 McFarland standard, as described in [4.4.2](#) for the broth culture method.

For all micro-organisms, the number of viable cells (in CFU/ml) in the final inoculum depends on the growth phase of the culture. This effect is most pronounced for fastidious organisms such as *Streptococcus pneumoniae*, where use of older cultures can significantly reduce the number of viable cells in the suspension.

A correctly adjusted suspension prepared by either method contains approximately 1×10^8 CFU/ml to 2×10^8 CFU/ml for the common relevant bacteria.

The adjusted inoculum prepared as above is diluted in broth to give a final cell number of 5×10^5 CFU/ml (target range 2×10^5 CFU/ml to 8×10^5 CFU/ml). The dilution required depends upon the bacterial species being tested and the method used for inoculum delivery. Transfer of 0,1 ml of standardized micro-organism suspension to a tube containing 9,9 ml (1:100 dilution) of broth results in a suspension of 1×10^6 CFU/ml which, when 50 μl is added to an equal volume (50 μl) of antimicrobial agent solution, results in a final inoculum of 5×10^5 CFU/ml with many Gram-negative bacteria (e.g. *Escherichia coli*). If the wells already contain 100 μl of antimicrobial agents in broth, an appropriate dilution to give the required final inoculum should be prepared prior to addition of up to 10 μl of the diluted suspension to each well. Different dilutions of the 0,5 McFarland suspension may be necessary, as determined by colony counts in preliminary tests^[5].

4.5 Inoculation of micro-dilution trays

The trays shall be inoculated within 30 min of standardizing the inoculum suspension, in order to maintain viable cell number in CFU/ml. To each well containing 50 μl of diluted antimicrobial agent in

broth (see 4.3), a volume of 50 µl of bacterial suspension (see 4.4) is added. For tray wells that contain 100 µl of diluted antimicrobial agent in broth, up to 10 µl of diluted inoculum suspension should be added.

Viable counts shall be performed on the test suspension to ensure that test wells contain $\sim 5 \times 10^5$ CFU/ml. This shall be done by removing 10 µl from the growth control well immediately after inoculation and diluting it in 10 ml of broth or saline. 100 µl of this dilution is spread over the surface of a suitable agar plate, which is then incubated overnight (12 h to 18 h). Twenty to eighty colonies would be expected from an acceptable test suspension. If this is not achieved, corrective action should be taken to ensure proper inoculum preparation.

4.6 Incubation of micro-dilution trays

Micro-dilution trays should be sealed in polyethylene bags or fitted with a tight lid or adhesive seal before incubation, in order to prevent desiccation. To avoid uneven heating, micro-dilution trays should not be stacked more than four high, and a single tight lid should be placed on top of the stack of test trays.

Unless otherwise specified, micro-dilution trays are incubated at $(35 \pm 1)^\circ\text{C}$ in ambient air for (18 ± 2) h for most antimicrobial agent-bacteria combinations. A CO_2 -enriched atmosphere should not be used.

4.7 Reading results

Results shall only be read when there is sufficient growth of the test organism (i.e. obvious button or definite turbidity in the positive growth control), when there is no growth in the un-inoculated or negative growth control (where present) and when purity and the appropriate cell number concentration of the inoculum has been established. The amount of growth in each well is compared with that in the positive growth control, and the MIC recorded is the lowest concentration of the agent that completely inhibits visible growth. There are exceptions to this (e.g., trailing endpoints for linezolid, partial inhibition by sulphonamides, incomplete inhibition with some bacteriostatic agents) that will require special attention by the user.

4.8 Special test situations where the MIC result might give unreliable results

In some cases, the MIC value may not reflect relevant activity. Therefore, the interpretations of test results with some antimicrobial agents may need to be modified for clinical application. In those situations, the reference method shall be modified e.g. by changes in incubation conditions or adjustments to the media. In addition, certain resistance mechanisms may not always be expressed using the standard reference dilution method, e.g. the expression of some β -lactamases, efflux pumps or drug target site modifications. In those cases, the MIC should be interpreted with caution, or other information used instead, to guide clinical therapy. In [Annex D](#) several antimicrobial agent/bacteria combinations are listed that require special attention.

5 Quality control

The quality of test results is monitored by the concomitant use of control strains. Stock control strains should be stored lyophilised or frozen (at -60°C or below). Prepare working cultures by subculture of stock control strains on a non-selective nutritive agar medium. Further subcultures may be made, from the first working culture only, for up to one week. When available, at least two relevant QC strains should be tested every day that testing is carried out. Test colonies of control cultures are processed in the same way as routine cultures. MICs of antimicrobial agents for control organisms should be within the ranges given in the latest versions of the CLSI M100 document^[15] or the EUCAST Quality Control document^[16]. It is not possible to provide a single Quality Control Table. Differences in media, reference studies, manufacturers of powders, etc. may create some differences in QC ranges that may affect laboratory test results. Further, quality control ranges may be adjusted over time as new information becomes available that are not reflected in this document. Finally, new agents may be added that are not included in this document. Standardization of the performance of broth micro-dilution antimicrobial susceptibility should limit most of the differences in quality control ranges, but laboratorians and

manufacturers of micro-dilution devices should refer to the identified documents to ensure that the ranges specified are adhered to.

Annex A **(informative)**

Requirements for Mueller-Hinton broth

A.1 General

Supplements other than divalent cations or other additional components should not be used unless necessary for growth of the test organism.

A.2 Testing of non-fastidious organisms in Mueller-Hinton broth

A.2.1 General

The standard medium for testing of non-fastidious organisms is Mueller-Hinton broth. The original ingredients in the formulation were as follows^[6]:

- Dehydrated infusion from 300 g beef;
- Acid digest of casein 17,5 g;
- Corn starch 1,5 g;
- QSP distilled water 1 000 ml;
- pH 7,2 to 7,4.

Almost all Mueller-Hinton broth is prepared commercially by a variety of manufacturers. The preparation of the broth from powder by all these manufacturers is similar and follows the original formulation of the medium.

A.2.2 Cation adjustment and content

The broth should contain sufficient concentrations of cations to provide adequate growth, and to permit the user to determine MIC values for quality control strains within ranges identified in CLSI and EUCAST documents.

New lots of Mueller-Hinton broth may require testing for acceptable cation content. This may be accomplished by either Inductively Coupled Plasma (ICP) spectroscopy or Flame Atomic Absorption Spectrometry (FAAS)^[7].

For calcium and magnesium ion adjustment, prepare 10 mg/l solutions of calcium chloride (3,68 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 ml deionised water) and magnesium chloride (8,36 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in 100 ml deionised water), sterilise by membrane filter and store at 2 °C to 8 °C. Each 0,1 ml volume of 10 mg/l cation solution added to 1 l of broth increases the cation content by 1 mg/l. Add whilst stirring at 2 °C to 8 °C.

For most antimicrobial agent/bacteria combinations, addition of calcium and magnesium to a final concentration of 20 mg/l to 25 mg/l, and of 10 mg/l to 12,5 mg/l, respectively, has been shown to provide accurate quality control results^{[8][9]}. Throughout this document reference to testing in Mueller-Hinton broth implies cation-adjusted Mueller-Hinton broth.

Situations in which additional adjustment of cation content to Mueller-Hinton broth is required for reproducible antimicrobial susceptibility testing are referenced below in this annex.

A.2.3 Medium adjustments to reference Mueller-Hinton broth micro-dilution medium for testing certain bacterial species and other microbial agents

A.2.3.1 *Streptococcus* species

Lysed horse blood should be added to Mueller-Hinton broth medium to a final volume concentration of 2,5 % to 5 % for testing of *Streptococcus* species. The blood should be obtained from a reputable and reliable supplier. Haematocrit information should be available (not less than 30 %). To prepare the lysed blood, aseptically mix equal volumes of defibrinated blood and sterile distilled water. Freeze at -20 °C and thaw until cells are thoroughly lysed (may require five to seven cycles). Clarify the solution by centrifugation. A clear solution is essential for reading. Failure to clarify the solution may be due to inadequate lysis or centrifugation. Repeating the centrifugation may improve the clarity of the solution. The resulting preparation of lysed blood is a 50 % volume concentration stock solution^[10].

A.2.3.2 Sulphonamides and trimethoprim

The medium shall have a thymidine mass concentration of less than 0,03 mg/l. Such a medium does not preclude use for testing of other antimicrobial agents^[11].

A.2.3.3 Tigecycline

Tigecycline shall be tested within 12 h of preparation of the Mueller-Hinton broth. Once prepared, the micro-dilution trays shall be frozen unless they are used fresh within the specified 12 h^[12].

A.2.3.4 Lipoglycopeptides (dalbavancin, telavancin and oritavancin)

For testing dalbavancin, telavancin and oritavancin, polysorbate-80 (volume fraction 0,002 %) should be added to Mueller-Hinton broth^[13].

A.2.3.5 Cefiderocol

For testing this siderophore cephalosporin agent, Mueller-Hinton broth is first depleted of iron with an iron chelating compound. The medium is then supplemented back with standard concentrations of calcium, magnesium and zinc^[14].

A.2.3.6 Oxacillin testing for Staphylococci

Broth micro-dilution may not reliably detect resistance conferred by the *mecA* or *mecC* gene. The following modifications of the test should be used, to enhance the detection of resistance:

- Incorporation of NaCl at a final concentration of 20 g/l in the broth when testing oxacillin.
- Incubation of tests for a full 24 h.
- Incubation temperature of maximum 35 °C.
- Use of the direct suspension method for preparing bacterial inocula

For certain strain types, even when using these conditions, the expression of the resistance is so low, that it is not reflected in the obtained MICs. This can in particular be seen for *mecC* positive isolates. Detection of the *mecA* or *mecC* gene is the reference method for detection of oxacillin resistance.

A.2.3.7 Daptomycin

Mueller-Hinton broth medium shall be supplemented to a final concentration of 50 mg/l Ca⁺⁺^[18].

A.2.3.8 Carbapenems

For the carbapenem agents, imipenem and meropenem, it has been shown that the final zinc concentration should be less than 3 mg/l^[19]. Mass concentrations of zinc required for optimal activity of other carbapenems have not yet been documented, but should be in the same range.

A.2.3.9 Glycopeptides

The MIC should be read after 24 h incubation to give more consistent and reliable results.

A.2.3.10 Polymyxins (Colistin)

Although it has been common practice in the past, polysorbate-80 or other surfactants should not be added to the medium when testing the agents in the polymyxin class, colistin and polymyxin B.

A.2.4 Supplementary medium issues for which international standardization is lacking

A.2.4.1 General

It is not possible at this time to identify all possibilities that may occur for quality control and reference antimicrobial susceptibility testing of aerobic and facultative anaerobic bacterial species. Some data on medium effects are contained in non-peer reviewed or manufacturer material. New agents will challenge the “standard” preparations of the medium. Users should ensure that standard quality control parameters are met and that new issues are communicated internationally, so that this document can be updated regularly. Known specific issues (e.g. testing of fastidious bacterial species, or specific additional requirements for standard Mueller-Hinton broth where the media differ in various parts of the world) are included in [Annex D](#) to make the reader aware of those testing issues.

NOTE For other new agents not included in this current document the reader is referred to the most recent CLSI^[15] and EUCAST^{[16][17]} document versions and to manufacturer information.

Annex B (informative)

Solvents and diluents for making stock solutions of selected antimicrobial agents

Antimicrobial agent	Solvent	Diluent
Amikacin	Water	Water
Amoxicillin	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Ampicillin	0,1 mol/l phosphate buffer, pH 8,0	0,1 mol/l phosphate buffer, pH 6,0
Arbekacin	Water	Water
Avibactam	Water	Water
Azithromycin	Ethanol volume fraction 95 % or glacial acetic acid ^a	Broth
Azlocillin	Water	Water
Aztreonam	Saturated sodium bicarbonate solution	Water
Besifloxacin	Methanol	Water
Biapenem	NaCl mass fraction 0,85 %	NaCl mass fraction 0,85 %
Cadazolid	DMSO	Water or broth
Carbenicillin	Water	Water
Cefaclor	Water	Water
Cefadroxil	0,1 mol/l phosphate buffer, pH 6,0	Water
Cefamandole	Water	Water
Cefazolin	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Cefcapene	0,1 mol/l phosphate buffer, pH 7,0	0,1 mol/l phosphate buffer, pH 7,0
Cefdinir	0,1 mol/l phosphate buffer, pH 6,0	Water
Cefditoren	0,1 mol/l phosphate buffer, pH 6,0	Water
Cefepime	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Cefetamet	0,1 mol/l phosphate buffer, pH 6,0	Water
Cefiderocol	Saline	Water
Cefixime	0,1 mol/l phosphate buffer, pH 7,0	0,1 mol/l phosphate buffer, pH 7,0
Cefmenoxime	0,1 mol/l phosphate buffer, pH 6,8	0,1 mol/l phosphate buffer, pH 6,8
Cefmetazole	Water	Water
Cefodizime	Water	Water
Cefonicid	Water	Water
Cefoperazone	Water	Water
Cefotaxime	Water	Water
Cefotetan	DMSO	Water
Cefotiam	Water	Water
Cefoxitin	Water	Water
Cefozopran	0,1 mol/l phosphate buffer, pH 7,0	0,1 mol/l phosphate buffer, pH 7,0
Cefpirome	Water	Water
Cefpodoxime	Mass concentration 0,1 % sodium bicarbonate solution	Water

Antimicrobial agent	Solvent	Diluent
Cefroxadine	0,1 N HCl	0,1 mol/l phosphate buffer, pH 6,0
Cefprozil	Water	Water
Ceftarolline	DMSO to 30 % of total volume	Saline
Ceftazidime	Saturated sodium bicarbonate solution ^b	Water
Cefteram	1/15 mol/l phosphate buffer, pH 7,0	1/15 mol/l phosphate buffer, pH 7,0
Ceftibuten	1/10 volume of dimethyl sulfoxide	Water
Ceftizoxime	Water	Water
Ceftobiprole	DMSO plus glacial acetic acid ^c	Water, vortex vigorously
Ceftolozane	Water or saline	Water or saline
Ceftriaxone	Water	Water
Cefuroxime	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Cephalothin	0,1 mol/l phosphate buffer, pH 6,0	Water
Cephalexin	0,1 mol/l phosphate buffer, pH 6,0	Water
Cephapirin	0,1 mol/l phosphate buffer, pH 6,0	Water
Cephradine	0,1 mol/l phosphate buffer, pH 6,0	Water
Chloramphenicol	Ethanol volume fraction 95 %	Water
Cinoxacin	1/2 volume of water, then add 1 mol/l NaOH to dissolve	Water
Ciprofloxacin	Water	Water
Clarithromycin	Methanol or glacial acetic acid ^a	0,1 mol/l phosphate buffer, pH 6,5
Clavulanic acid	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Clinafloxacin	Water	Water
Cloxacillin	Water	Water
Clindamycin	Water	Water
Colistin ^d	Water	Water
Dalbavancin	DMSO	Water and DMSO ^e
Daptomycin	Water	Water
Delafoxacin	1/2 volume of water, then 0,1 mol/l NaOH dropwise to dissolve	Water
Dibekacin	Water	Water
Dirithromycin	Glacial acetic acid ^a	Water
Doripenem	NaCl mass fraction 0,85 %	NaCl mass fraction 0,85 %
Doxycycline	Water	Water
Enoxacin	1/2 volume water, a minimum volume 0,1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Eravacycline	Water	Water
Ertapenem	0,01 mol/l phosphate buffer, pH 7,2	0,01 mol/l phosphate buffer, pH 7,2
Erythromycin	Ethanol volume fraction 95 % or glacial acetic acid ^a	Water
Faropenem	Water	Water
Fidaxomicin	DMSO	Water
Finafloxacin	Water	Water
Fleroxacin	1/2 volume water, a minimum volume 0,1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Flomoxef	Water	Water

Antimicrobial agent	Solvent	Diluent
Flucloxacillin	0,05 mol/l phosphate buffer, pH 7,0	Water
Fosfomycin	Water	Water
Fusidic acid	Water	Water
Garenoxacin	Water (with stirring)	Water
Gatifloxacin	Water (with stirring)	Water
Gemifloxacin	Water	Water
Gentamicin	Water	Water
Gepotidacin	DMSO	Water
Grepafloxacin	Water and 0,1 mol/l NaOH dropwise to dissolve	Water
Iclaprim	DMSO	Water
Isepamicin	Water	Water
Imipenem	0,01 mol/l phosphate buffer, pH 7,2	0,01 mol/l phosphate buffer, pH 7,2
Josamycin	Methanol	Water
Kanamycin	Water	Water
Lefamulin	Water	Water
Levofloxacin	1/2 volume water, a minimum volume 1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Levonadifloxacin	27,5 mg/l solution of L-arginine in water	Water
Lincomycin	Water	Water
Linezolid	Water	Water
Linopristin-flopristin	Dimethylformamide (DMF) to 25 % of final volume/ water for solvent	Water
Lomefloxacin	Water	Water
Loracarbef	Water	Water
Mecillinam	Water	Water
Meropenem	Water	Water
Methicillin	Water	Water
Metronidazole	DMSO	Water
Mezlocillin	Water	Water
Minocycline	Water	Water
Moxalactam (diammonium salt) ^f	0,04 mol/l HCl (let sit for 1,5 h to 2 h)	0,1 mol/l phosphate buffer, pH 6,0
Moxifloxacin	Water	Water
Mupirocin	Water	Water
Nacubactam	Water	Water
Nafcillin	Water	Water
Nafithromycin	1/2 volume of water, then glacial acetic acid dropwise to dissolve (acetic acid not to exceed 2,5 mg/l)	Water
Nalidixic acid	1/2 volume water, a minimum volume 1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Netilmicin	Water	Water
Nitazoxanide	DMSO	Water
Nitroxoline	DMSO	Water
Nitrofurantoin	0,1 mol/l phosphate buffer, pH 8,0; alternatively DMSO	0,1 mol/l phosphate buffer, pH 8,0

Antimicrobial agent	Solvent	Diluent
Norfloxacin	1/2 volume of water, a minimum volume 0,1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Ofloxacin	1/2 volume water, a minimum volume 0,1 mol/l NaOH to dissolve, then make up to total volume with water	Water
Omadacycline	Water	Water
Oritavancin	0,002 % polysorbate-80 in water	0,002 % polysorbate-80 in water
Oxacillin	Water	Water
Ozenoxacin	0,1 mol/l NaOH	Water
Panipenem	Water	Water
Pazufloxacin	Water	Water
Pexiganan	Water	Water
Penicillin	Water	Water
Piperacillin	Water	Water
Plazomicin	Water	Water
Polymyxin B	Water	Water
Prulifloxacin	DMSO	Water
Quinupristin-dalfopristin	Water	Water
Ramoplanin	Water	Water
Razupenem	0,01 mol/l phosphate buffer, pH 7,2	0,01 mol/l phosphate buffer, pH 7,2
Relebactam	Water	Water
Rifampicin	Methanol	Water
Rifaximin	Methanol	0,1mol/l phosphate buffer pH 7,4 + 0,45 % sodium dodecyl sulfonate
Roxithromycin	Methanol	0,1 mol/l phosphate buffer, pH 8,0
Secnidazole	DMSO	Water
Sitafloxacin	0,1 mol/l NaOH	Water
Solithromycin	Glacial acetic acid	Water
Sparfloxacin	Water	Water
Spectinomycin	Water	Water
Streptomycin	Water	Water
Sulbactam	Water	Water
Sulphonamides	Minimum volume 2,5 mol/l NaOH to dissolve, then make up to total volume with water	Water
Sulfamethoxazole	1/2 volume hot water and minimal amount of 2,5 mol/l NaOH to dissolve	Water
Sulfisoxazole	1/2 volume hot water and minimal amount of 2,5 mol/l NaOH to dissolve	Water
Sultamicillin	Methanol	Water
Sulopenem	0,01 mol/l phosphate buffer, pH 7,2, vortex to dissolve	0,01 mol/l phosphate buffer, pH 7,2
Surotomycin	Water	Water
Tazobactam	Water	Water
Tebipenem	Water	Water

Antimicrobial agent	Solvent	Diluent
Tedizolid	DMSO ^g	DMSO
Teicoplanin	Water	Water
Telavancin	DMSO	DMSO
Telithromycin	Glacial acetic acid ^a	Water
Temocillin	Water	Water
Tetracycline	Water	Water
Ticarcillin	0,1 mol/l phosphate buffer, pH 6,0	0,1 mol/l phosphate buffer, pH 6,0
Tigecycline	Water	Water
Tinidazole	DMSO	Water
Tizoxanide	DMSO	DMSO
Tobramycin	Water	Water
Tosufloxacin	0,1 mol/l NaOH	Water
Trimethoprim	0,05 mol/l lactic acid or 0,05 mol/l HCl, 10 % of final volume with water	Water
Trimethoprim (if lactate)	Water	Water
Trospectomycin	Water	Water
Vaborbactam	DMSO	Water
Vancomycin	Water	Water

NOTE 1 The information regarding solvents and diluents in [Annex B](#) was largely obtained from CLSI document M100 28th ed. Reprinted with permission from Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018^[15]. This information is subject to periodic updates. Check the latest version of M100 available from CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087, USA.

NOTE 2 For further information on examples of solvents and diluents for making stock solutions of selected antimicrobial agents, consult the European Pharmacopoeia or the US Pharmacopoeia.

^a For glacial acetic acid, use half volume of water, then add glacial acetic acid dropwise until dissolved, not to exceed 2,5 mg/l; add water to full volume. Glacial acetic acid is equivalent to acetic acid volume fraction >99 %.

^b Anhydrous sodium carbonate is used at a mass of exactly 10 % of the ceftazidime to be used. The sodium carbonate is dissolved in solution in most of the necessary water. The antimicrobial agent is dissolved in this sodium carbonate solution, and water is added to the desired volume. The solution is to be used as soon as possible, but it can be stored up to 6 h at not more than 25 °C.

^c For each 1,5 mg ceftobiprole, add 110 µl of a 10:1 mixture of dimethyl sulfoxide and glacial acetic acid. Vortex vigorously for 1 min, then intermittently for 15 min. Dilute to 1,0 ml with distilled water.

^d The formulation of colistin used in antimicrobial susceptibility tests is colistin sulphate and not colistin methane sulphonate (sulphomethate).

^e Starting stock solutions of dalbavancin should be prepared at concentrations no higher than 1,600 mg/l. Intermediate ×100 concentrations should then be diluted in dimethyl sulfoxide (DMSO). Final 1:100 dilutions should then be made directly into Mueller-Hinton broth supplemented with polysorbate-80 volume fraction 0,002 % so that the final concentration of DMSO in the wells is no greater than 1 %.

^f The diammonium salt of moxalactam is very stable, but it is almost pure R isomer. Moxalactam for clinical use is a 1:1 mixture of R and S isomers. Therefore, the salt is dissolved in 0,04 mol/l HCl and allowed to react for 1,5 h to 2 h to convert it to equal parts of both isomers.

^g Starting stock solutions of tedizolid should be prepared at concentrations no higher than 1,600 mg/l. Intermediate ×100 concentrations should be diluted in DMSO. Final 1:100 dilutions should be made directly into CAMHB so that the final concentration of DMSO in the wells is not greater than 1 % for diluent.

Annex C (informative)

Preparation of working dilutions of antimicrobial agents for use in broth dilution susceptibility tests

Antimicrobial agent concentration in stock solution mg/l	Volume stock solution ml	Volume broth ^a ml	Antimicrobial agent concentration obtained mg/l
512 0	1	9	512
512	1	1	256
512	1	3	128
512	1	7	64
64	1	1	32
64	1	3	16
64	1	7	8
8	1	1	4
8	1	3	2
8	1	7	1
1	1	1	0,5
1	1	3	0,25
1	1	7	0,125

^a Broth used for dilution is that used in the susceptibility test. Any supplementation shall take place before diluting the antimicrobial agent to maintain the required concentrations. Preparation of working dilutions from Reference [8].

Annex D **(informative)**

Special test situations

D.1 General

This annex contains a number of test situations that, when performed by broth micro-dilution have not been fully standardised internationally, or where Mueller-Hinton broth micro-dilution testing may not provide reliable results. Nevertheless, it is important for laboratories to be aware of these situations so that data can be gathered to eventually incorporate them into the body of the international standard. In all cases, Mueller-Hinton medium is used as the base for testing these agents. There may be other test situations that arise over time that will require validation to place them in an international standard.

D.2 Special test situations

D.2.1 Fastidious aerobic and facultative anaerobic bacterial species (e.g. Haemophilus)

Two methods are currently used and have not been fully compared. Haemophilus Test Medium is documented by CLSI^[1], and Mueller-Hinton Fastidious (MH-F) Broth is documented by EUCAST^[16]. Readers are referred to the most recent versions of CLSI and EUCAST documents for information on medium preparation, testing and quality control results. When comparative testing of these methods has been completed and validated, this document will be updated to include reference antimicrobial susceptibility test method(s) for these micro-organisms.

D.2.2 Mecillinam

Studies have shown that testing of mecillinam by Mueller-Hinton broth micro-dilution results in severe trailing endpoints and is therefore not recommended^[20]. Agar dilution or disc diffusion provide stable reproducible results.

D.2.3 Fosfomycin

Broth micro-dilution may not give reliable results. Agar dilution should be used as the reference method. The test agar should be supplemented with 25 mg/l glucose-6-phosphate.

Bibliography

- [1] Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically 7th ed. *Approved Standard M7-A7*, Clinical and Laboratory Standards Institute Wayne, PA [2006]
- [2] Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth microdilution. European Committee on Antimicrobial Susceptibility Testing. EUCAST Discussion Document E.Def 5.1. *Clin Microbiol Infect*, (2003), vol 9 (issue 7 insert). pp. 1–10 (see http://www.eucast.org/documents/publications_in_journals/)
- [3] ERICSSON H.M., SHERRIS J.C., Antibiotic sensitivity testing. Report of an international collaborative study, *Acta Pathol Microbiol Scand*. (1971), sect. B 217 (suppl). pp. 1–90
- [4] MCFARLAND J., The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J. Amer Med Assoc*. (1907), vol 49. pp. 1176–1178
- [5] MOOSDEEN F., WILLIAMS J.D., SECKER A, Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. *J Antimicrob Chemother*. (1988), vol. 21. pp. 439–443
- [6] MUELLER J.H., HINTON J, A protein-free medium for primary isolation of the gonococcus and meningococcus. *Proc Soc Exper Biol Med*. (1941), vol. 48. p. 330
- [7] MORRISON G.H., In *Critical Reviews in Analytical Chemistry*. (1969), vol. 14(28A), CRC Press, Boca Raton, Florida
- [8] BARRY A.L., MILLER G.H., THORNSBERRY C. et al. , Influence of cation supplements on activity of netilmicin against *Pseudomonas aeruginosa* in vitro and in vivo. *Antimicrob Agents Chemother*. (1987), vol 31. pp. 1514–1518
- [9] BARRY A.L., RELLER L.B., MILLER G.H. et al. , Revision of standards for adjusting the cation content of Mueller-Hinton broth for testing susceptibility of *Pseudomonas aeruginosa* to aminoglycosides. *J. Clin Microbiol*. (1992), vol. 30. pp. 585–589
- [10] D'AMATO R.F., SWENSEN J.M., MCKINLEY G.A. et al. , Quantitative antimicrobial susceptibility test for *Streptococcus pneumoniae* using inoculum supplemented with whole defibrinated sheep blood. *J Clin Microbiol*. (1987), vol. 25. pp. 1753–1756
- [11] SWENSEN J.M., THORNSBERRY C., Susceptibility tests for sulfamethoxazole-trimethoprim by broth microdilution procedure. *Curr Microbiol*. (1978), vol. 1. pp. 189–193
- [12] BRADFORD P.A., PETERSEN P.J., YOUNG M. et al. , Tigecycline MIC testing by broth dilution requires Use of fresh medium or addition of the biocatalytic oxygen-reducing reagent oxyrase to standardize the test method. *Antimicrob Agents Chemother*. (2005), vol. 49. pp. 3903–3909
- [13] RENNIE R.P., KOETH L., JONES R.N. et al. , Factors influencing broth microdilution antimicrobial susceptibility test results for dalbavancin, a new glycopeptide agent. *J. Clin. Microbiol*. (2007), vol. 45. pp. 3151–3154
- [14] AKINOBU I., TORU NISHIKAWA T., MATSUMOTO S. et al. , Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. (2016), vol. 60. pp. 7396–7401
- [15] Performance Standards for Antimicrobial Susceptibility Testing 28th ed. *Informational Supplement M100-S28*, Wayne, PA Clinical and Laboratory Standards Institute (2018)

- [16] Routine internal quality control as recommended by EUCAST European Committee on Antimicrobial Susceptibility Testing (EUCAST). (for the latest version see; http://www.eucast.org/ast_of_bacteria/qc_tables)
- [17] Media preparation for EUCAST disc diffusion testing and for determination of MIC values by the broth microdilution method. European Committee on Antimicrobial Susceptibility Testing,(EUCAST). (for the latest version see: http://www.eucast.org/ast_of_bacteria/media_preparation/)
- [18] FUCHS P.C., BARRY A.L., BROWN S.D, Daptomycin susceptibility tests: interpretive criteria, quality control and effect of calcium on in vitro tests. *Diag Microbiol Infect Dis.* (2000). vol. **38**. pp. 51–58
- [19] DALY J.S., DODGE R.A., GLEW R.H. et al. , Effect of zinc concentration in Mueller-Hinton agar on susceptibility of *Pseudomonas aeruginosa* to imipenem. *J. Clin Microbiol.* (1997), vol. **35**. pp. 1027–1029
- [20] SKOV R., FRIMOT-MOLLER N., MENDAY P. et al. , Susceptibility testing of urinary isolates of *Escherichia coli* to mecillinam using NCCLS methodology. *International J Antimicrob Agents.* (2005), vol. **25**. pp. 198-202