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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 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

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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.

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 ≤200 µl), 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].

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 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 ≤200 µl 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 µl per well with double the desired final concentrations of antimicrobial agent, or at 100 µl per well in the desired final concentrations.

At least one well, containing 50 µl or 100 µl of antimicrobial agent-free medium, should be included as a growth control for each strain tested. Likewise, a well containing 100 µl 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 ± 1) °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 $BaCl_2 \cdot 2H_2O$) 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 ± 1 °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 ~ 5×10^5 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 to18 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 ± 1) °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 $CaCl_2 \cdot 2H_2O$ in 100 ml deionised water) and magnesium chloride (8,36 g $MgCl_2 \cdot 6H_2O$ 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, televancin and oritivancin)

For testing dalbavancin, televancin and oritivancin, 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
Delafloxacin	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 drop-wise 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