INTERNATIONAL STANDARD

**ISO 20776-2**

Second edition
2021-12

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

Part 2:

# Evaluation of performance of antimicrobial susceptibility test devices against reference broth microdilution



*Systèmes d'essais en laboratoire et de diagnostic in vitro — Sensibilité in vitro des agents infectieux et évaluation des performances des dispositifs pour antibiogrammes —*

*Partie 2: Évaluation des performances des dispositifs pour antibiogrammes par rapport à une méthode de référence de microdilution en bouillon*

Reference number
ISO 20776-2:2021(E)



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

Published in Switzerland

# Contents

Page

**Foreword** ... **iv**
**Introduction** ... **vi**
**1 Scope** ... **1**
**2 Normative references** ... **1**
**3 Terms and definitions** ... **1**
**4 Test methods** ... **5**
4.1 General ... 5
4.2 Methods ... 5
4.2.1 Reference method ... 5
4.2.2 Strain selection ... 5
4.2.3 Quality control (QC) ... 6
4.2.4 Quality control (QC) of the reference method ... 6
4.2.5 Reproducibility testing of test device ... 6
4.2.6 Isolate testing protocol ... 7
4.2.7 Inoculum preparation ... 7
4.2.8 Discrepancy resolution testing ... 7
4.2.9 System under evaluation ... 7
**5 Data analysis and acceptance criteria** ... **8**
5.1 Accuracy of test device ... 8
5.1.1 General ... 8
5.1.2 MIC devices ... 8
5.1.3 Qualitative AST devices ... 8
5.2 Quality control (QC) of test device ... 8
5.3 Reproducibility of test device ... 8
5.4 Documents related to study ... 8
**Annex A** (informative) **Evaluating the performance of MIC tests** ... **9**
**Annex B** (informative) **Rationale for bias analysis** ... **12**
**Annex C** (informative) **Sensitivity and specificity analyses for qualitative tests** ... **14**
**Bibliography** ... **16**

# 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,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, *In vitro diagnostic medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).



This second edition cancels and replaces the first edition (ISO 20776-2:2007), which has been technically revised.

The main changes are as follows:

— Revision in the title of this document to better align with the intended information.

— Addition of an Introduction (not present in the first edition).

— Revised Clause 3 as follows:

  — Removed definitions for category agreement, susceptible, intermediate, resistant, non-susceptible, major discrepancy, minor discrepancy, very major discrepancy, breakpoint test and zone diameter;

  — Added definition for contemporary isolate (3.11.1), and removed definitions for fresh isolate, recent isolate;

  — Added definitions for reproducibility (3.9), bias of the test method (3.10.3), sensitivity analysis (3.10.4.1), specificity analysis (3.10.4.2), bacterial organism group (3.16);

  — Added definition for qualitative test (3.7) and removed definition for breakpoint test;

  — Revised definitions for minimum inhibitory concentration test (3.4), breakpoint (3.6), quality control (3.8), discrepancy (3.10.1).

— Reordered Clause 4 (Test methods);

— Moved general requirements for a performance evaluation as a separate section, to the overview (now renamed general section, subclause 4.1) under test methods);

— Revised quality control section, subclause 4.2, and referenced EUCAST and CLSI documents for quality control ranges;

— Revised subclause 4.2.1 (Reference method) to add variability;

— Revised subclause 4.2.2 (Strain selection) and incorporated new definition of contemporary isolates (3.11.1);

— Revised subclause 4.2.5 (Reproducibility testing);

— Updated subclause 4.2.8 (Discrepancy resolution testing);

— Combined data analysis and acceptance criteria subclauses (Clause 5);

— Revised subclause 5.1 (Accuracy of test device) to remove category agreement;

— Revised data analysis for MIC devices to remove category agreement. Added bias requirement;

— Removed acceptance for breakpoint AST devices;

— Added provisions on acceptance criteria for qualitative AST devices (5.1.3) and included sensitivity and specificity requirements;

— Revised subclauses on quality control of test device and reproducibility of test device (5.2 and 5.3);

— Revised Bibliography;

— Added Annex A — Evaluation the Performance of MIC Tests, Annex B — Rationale for Bias Analysis, and Annex C — Sensitivity and Specificity Analyses for Qualitative Tests.

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.

# Introduction

In vitro antimicrobial susceptibility tests are performed on bacteria suspected of causing disease, particularly if the isolate is thought to belong to a species that can 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 and 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 can be unreliable and when a quantitative result is required for clinical management. In dilution tests, bacterial strains 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 an isolated bacterial strain within a defined period of time, is known as the MIC. Careful control and standardization are required for intra- and interlaboratory reproducibility of broth MIC tests. The MICs of quality control (QC) strains generally span three doubling dilutions with a dominant central value, but can have a four-dilution range.

Broth micro-dilution denotes the performance of the broth dilution test in micro-dilution trays. Broth micro-dilution is now one of the most common methods used globally to perform antimicrobial susceptibility tests.



This document is the second edition of ISO 20776-2. It is designed for the evaluation of antimicrobial test devices against the standard broth micro-dilution reference method (ISO 20776-1) using 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 can need to be modified depending on the antimicrobial agent being tested.



Quantitative MIC and qualitative evaluations detailed in this revised document measure the accuracy, reproducibility and QC of tests performed with antimicrobial test devices that generate MIC values against the standard broth micro-dilution reference method. Antimicrobial agar disc diffusion tests are not included in this document.

This document has been revised using the premise that the MIC test is an in vitro assay, subject to intra- and interlaboratory assay variation. When making the comparison between any derivative test and that of the reference method, it is appropriate to apply measures of assay performance only and not result interpretation. For this reason, and because interpretive categories were removed from the second edition of ISO 20776-1, categorical agreement (CA) and its associated terminology, as described by the U.S. Food and Drug Administration (FDA), the Clinical and Laboratory Standards Institute (CLSI) M23 document, and other international documents, has not been applied. Avoiding an assessment of CA also assists in reducing the requirement to reassess assay performance automatically when the only change has been a breakpoint change (which is external to the assay itself).

This document applies to new performance evaluations initiated after the publication date; studies conducted prior to the acceptance date of this document should not need to be re-designed and/or re-analysed using these criteria. Studies conducted prior to these standards or acceptance of this document follow standard practice or guidance at the time of the study.

For derivative tests with more than three two-fold dilutions, assay performance is assessed with tools designed to measure accuracy using essential agreement (EA) and bias, and precision using EA only. For derivative tests with 1 to 3 concentrations, assay performance is assessed using standard sensitivity and specificity measures.

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

## Part 2:
## Evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution

## 1 Scope

This document establishes acceptable performance criteria for antimicrobial susceptibility test (AST) devices that are used to determine minimum inhibitory concentrations (MIC) of bacteria to antimicrobial agents in medical laboratories.

This document specifies requirements for AST devices and procedures for assessing performance of such devices. It defines how a performance evaluation of an AST device is to be conducted.

This document has been developed to guide manufacturers in the conduct of performance evaluation studies.



## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 20776-1, *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*

## 3 Terms and definitions

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

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

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

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

**3.1**
**antimicrobial susceptibility test device**
**AST device**
device, including all specified components used to obtain test results that allow MIC determination of bacteria with specific antimicrobial agents

Note 1 to entry: Specific components of the device include inoculators, disposables and reagents, media used to perform the test, and readers or analysers. Non-specific components, such as swabs, pipettes and tubes, are not part of the device.

**3.2**
**reference method**
method of analysis recognized by experts or used as a reference by agreement between parties, which gives, or is supposed to give the accepted reference value of the measurand

Note 1 to entry: For the purpose of this document, the reference method described in ISO 20776-1 is employed. This reference method describes dilution procedures to determine the *minimum inhibitory concentration* (3.3) of antimicrobial agents.

[SOURCE: ISO/TS 22176:2020, 3.1.20, modified — Note 1 to entry added.]

**3.3**
**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.

**3.4**
**minimum inhibitory concentration test**
**MIC test**
test that is capable of determining a *MIC* (3.3) covering a range of at least four consecutive doubling dilutions, and for which *essential agreement (EA)* (3.10.2) can be determined

**3.5**
**on-scale MIC test result**
**on-scale minimum inhibitory concentration (MIC) test result**
result from a *minimum inhibitory concentration (MIC) test* (3.4) when there is growth in at least one dilution below the MIC endpoint and no growth in at least one dilution above



**3.6**
**breakpoint**
specific values of parameters, such as *MICs* (3.3), on the basis of which bacteria can be assigned to clinical categories such as “susceptible” (S) or “resistant” (R)



Note 1 to entry: For current interpretive breakpoints and interpretive categories, reference should be made to the latest publications of organizations employing this *reference method* (3.2) (e.g. CLSI[2] and EUCAST[3]).

**3.7**
**qualitative test**
test for which the principal objective is to provide a qualitative result

EXAMPLE Using a *breakpoint* (3.6) or screening concentration.

Note 1 to entry: Such tests have a limited range of 1 to 3 doubling dilutions.

**3.8**
**quality control**
**QC**
use of carefully selected bacterial strains with given expected *minimum inhibitory concentration* (*MIC)* (3.3) results

Note 1 to entry: *MICs* of antimicrobial agents for control organisms should be within the ranges given in the latest editions of the CLSI M100 document[2] or the EUCAST Quality Control document.[4] It is not possible to provide a single Quality Control Table.

**3.9**
**reproducibility**
extent to which consistent results such as MICs are obtained when the test is repeated

### 3.10 Terms relating to the evaluation of test results

**3.10.1**
**discrepancy**
difference in a result between the test method [either a *minimum inhibitory concentration* (*MIC) test* (3.4)] or a *qualitative test* (3.7)) and the result of the *reference method* (3.2) outside the region of *essential agreement (EA)* (3.10.2) (*MIC test*), or outside the region of sensitivity and specificity (*qualitative test*)

**3.10.2**
**essential agreement**
**EA**
*minimum inhibitory concentration* (*MIC)* (3.3) result obtained with the *antimicrobial susceptibility test (AST) device* (3.1) that is within plus or minus one two-fold dilution step from the *MIC* value established with the *reference method* (3.2)

Note 1 to entry: Used for *MIC* devices.

Note 2 to entry: Another representation of the concept is:

$$\frac{N_{EA}}{N} * 100$$

where:

$N_{EA}$ is the number of bacterial isolates with an EA;

$N$ is the total number of bacterial isolates tested.

Note 3 to entry: The overall EA is expressed as a percentage.



**3.10.3**
**bias of the test method**
evaluation of test device results to determine whether the results that differ from the *reference method* (3.2) are significantly skewed or predominantly in one direction

Note 1 to entry: Used for *minimum inhibitory concentration (MIC) tests* (3.4).



**3.10.4 Terms relating to sensitivity analysis**

**3.10.4.1**
**sensitivity analysis**
<screening or *breakpoint* (3.6) test> measure of agreement between test device results and *reference method* (3.2) results that are positive or above a published *breakpoint* (3.6)

Note 1 to entry: This can also be considered as positive percent agreement when reference results are interpreted as positive.

Note 2 to entry: Used for *qualitative tests* (3.7). See Table 1.

**Table 1 — Sensitivity analysis for a qualitative (screening or breakpoint) test**

| | | Reference method | | Total |
|---|---|---|---|---|
| | | (−) or no growth | (+) or growth | |
| Test method | (−) or no growth | *a* | *b* | *a+b* |
| | (+) or growth | *c* | *d* | *c+d* |
| Total | | *a+c* | *b+d* | Sum of (*a,b,c,d*) |
| **Sensitivity =** $100*[d \div (b+d)]$ | | | | |

**3.10.4.2**
**sensitivity analysis**
<three-dilution *qualitative test* (3.7)> measure of agreement between test device results and *reference method* (3.2) results that have the *MICs* (3.3) at the high end of the scale

Note 1 to entry: Used for *qualitative tests* (3.7). See Table 2.

**Table 2 — Sensitivity analysis for a three-dilution qualitative test**

| | | Reference method | | | Total |
|---|---|---|---|---|---|
| | | ≤ Low MIC | Middle MIC | ≥ High MIC | |
| Test method | ≤ Low MIC | *a* | *b* | *c* | *a+b+c* |
| | Middle MIC | *d* | *e* | *f* | *d+e+f* |
| | ≥ High MIC | *g* | *h* | *i* | *g+h+i* |
| Total | | *a+d+g* | *b+e+h* | *c+f+i* | Sum of (*a* to *i*) |
| **Sensitivity** = $100*[i \div (c+f+i)]$ | | | | | |

## 3.11 Terms relating to bacterial isolates

**3.11.1**
**contemporary isolate**
isolate recovered from a clinical sample within the previous six months that has been minimally sub-cultured

Note 1 to entry: Ideally, they are consecutive and prospectively collected. These isolates can have been frozen prior to use.

**3.11.2**
**stock isolate**
isolate recovered from a clinical sample that has been retained, stored or obtained from a culture collection

Note 1 to entry: Stock isolates are usually included because they have known or rare resistance mechanisms or are of a genus or species for which the antimicrobial agent is indicated but are not commonly isolated. Such organisms are unlikely to be available in *contemporary isolates* (3.11.1) used in the evaluation. There is no requirement for how long ago the isolate was obtained.

**3.12**
**coordinator**
person empowered by the manufacturer or *investigator* (3.13) with responsibility for the entire performance evaluation

**3.13**
**investigator**
person responsible for the execution of the performance evaluation at a certain location

**3.14**
**evaluation plan**
description of a planned performance evaluation

**3.15**
**evaluation report**
description of and conclusions from a performance evaluation

**3.16**
**bacterial organism group**
group of related bacterial genera and species that share similar characteristics

# 4 Test methods

## 4.1 General

The manufacturer or investigator takes the responsibility for the initiation and the conduct of a performance evaluation according to the evaluation plan. The manufacturer shall define the responsibility and the interrelation of all personnel who manage and conduct a performance evaluation.

The manufacturer or investigator shall appoint a coordinator with overall responsibility for the performance evaluation and the evaluation report. This may include a coordinator, who shall assess and document criteria used and indicate which performance claims are met.

An evaluation conducted by a manufacturer shall consist of accuracy using contemporary and stock strains, reproducibility and quality control (QC) testing performed in at least three different laboratories, of which a maximum of one can be the manufacturer's laboratory. Alternatively, these studies may be conducted at a single site that mimics three sites (e.g. multiple users/instruments, geographically diverse source of organisms). This single site can be within the manufacturer's laboratory. The complete testing protocol should focus on the most commonly used manufacturer's instructions for use or primary methods.

Alternatively, studies incorporating variations of the manufacturer's instructions for use (e.g. inoculation procedures or manual reads of the device) or other secondary methods should consist of the reproducibility and QC sections described below. These studies can be conducted at a single site that mimics three sites (e.g. multiple users/instruments, geographically diverse source of organisms). This single site can be within the manufacturer's laboratory.

Changes made to the test device to correspond to published breakpoint changes for one group of organisms (e.g. Gram-negative fermentative bacilli) or changes made to QC range changes should not require re-test or re-analysis of all bacterial isolates if those changes involve previously validated concentrations in the test device. Initial evaluation of test devices can be performed with longer dilution sequences than used on final products, to provide a comprehensive evaluation while giving flexibility in dilutions provided on product for routine use.



## 4.2 Methods

### 4.2.1 Reference method

The reference method shall be as described by ISO 20776-1.

The reference method procedure can be performed either simultaneously with the test device at all testing sites, or at a single site for all isolates tested in the study, which can be that of the manufacturer. If the reference method and test device are tested at the same site, the reference method and the test device shall be set up on the same day from the same inoculum source.

The variability of the reference method can be determined prior to or during AST device studies. A single reference method test point can be used. If reference variability is noted, the results should indicate either the need to perform reference testing at a single site to eliminate variability, or to perform replicate testing of the reference method, in which case the mode or median value of the replicate tests is used.

NOTE In some cases, results from other widely accepted methods can be used in addition to the reference MIC result to arbitrate results. For example, tests that detect the presence of a specific resistance gene, such as the *mecA* gene (encoding oxacillin resistance) or the gene product (PBP 2a), are widely employed and are considered reference methods for detecting oxacillin resistance in staphylococci.

### 4.2.2 Strain selection

An evaluation protocol should incorporate at least 300 clinical isolates overall (100/site) relevant to an antimicrobial agent. Only one isolate per species per patient shall be included. The collection should