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

Part 2: Evaluation of performance of antimicrobial susceptibility test devices

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

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Foreword

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

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

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

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

ISO 20776-2 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, In vitro *diagnostic medical devices,* in collaboration with Technical Committee ISO/TC 212, *Clinical laboratory testing and* in vitro *diagnostic test systems*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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

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

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

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

Part 2: Evaluation of performance of antimicrobial susceptibility test devices

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1 Scope

This part of ISO 20776 establishes acceptable performance criteria for antimicrobial susceptibility test (AST) devices that are used to determine minimum inhibitory concentrations (MIC) and/or interpretive category determinations of susceptible, intermediate and resistant (SIR) strains of bacteria to antimicrobial agents in medical laboratories. This part of ISO 20776 specifies requirements for AST devices (including diffusion test systems) and procedures for assessing performance of such devices. It defines how a performance evaluation of an AST device is to be conducted. This part of ISO 20776 has been developed to guide manufacturers in the conduct of performance evaluation studies.

2 Normative references

The following referenced documents are indispensable for the application 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, *Clinical laboratory testing and* in vitro *diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: Reference method for testing the* in vitro *activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases*

3 Terms and definitions

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

3.1 Agreement of test results

3.1.1
category agreement
CA
agreement of SIR results between a breakpoint test or an MIC test and the reference method (ISO 20776-1)

Another representation of the concept:

$$\frac{N_{CA} \times 100}{N}$$

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where

N_{CA} is the number of bacterial isolates with the same SIR category as the reference method category result;

N is the total number of bacterial isolates tested

NOTE The overall CA is expressed as a percentage.

3.1.2
essential agreement
EA
MIC result obtained with the AST device that is within plus or minus one doubling dilution step from the MIC value established with the reference method (ISO 20776-1)

Another representation of the concept:

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

where

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

N is the total number of bacterial isolates tested

NOTE The overall EA is expressed as a percentage.

3.2
antimicrobial susceptibility test device
AST device
device including all specified components used to obtain test results that allow SIR categorization of bacteria with specific antimicrobial agents

NOTE Specific components include inoculators, disposables and reagents, media, disks and readers. Non-specific components, such as swabs, pipettes and tubes, are not part of the device.

3.3
breakpoint
BP
specific values of parameters, such as MICs, on the basis of which bacteria can be assigned to the clinical categories “susceptible”, “intermediate” and “resistant”

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

3.3.1
susceptible
S
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success

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

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

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3.3.2
intermediate
I
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with uncertain therapeutic effect

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

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

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

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

3.3.3
resistant
R
bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure

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

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

3.3.4
non-susceptible
NS
bacterial strain for which the test result exceeds the susceptible breakpoint and for which there are no established intermediate or resistant breakpoints

NOTE This is generally due to lack of strains with resistance to the antimicrobial agent when the breakpoints are defined.

3.4
breakpoint test
BPT
test that has the principal objective to provide categorical results (SIR)

NOTE This can include limited range dilution tests or diffusion tests.

3.5
coordinator
person empowered by the manufacturer or investigator with responsibility for the entire performance evaluation

3.6 Discrepancies

3.6.1
major discrepancy
MD
test result by the reference method interpreted as S and an AST device result of R

Another representation of the concept:

$$\frac{N_{\mathrm{MD}} \times 100}{N_{\mathrm{SREF}}}$$

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where

N_{MD} is the number of tests that result in a MD;

N_{SREF} is the number of susceptible bacterial isolates as determined by the reference method (ISO 20776-1)

NOTE The overall MD is expressed as a percentage.

3.6.2
minor discrepancy
mD
test result by the reference method interpreted as R or S and an AST device result of I; or a reference result interpreted as I and an AST device result of R or S

Another representation of the concept:

$$\frac{N_{mD} \times 100}{N}$$

where

N_{mD} is the number of tests that result in a mD;

N is the total number of bacterial isolates tested

NOTE The overall mD is expressed as a percentage.

3.6.3
very major discrepancy
VMD
test result by the reference method interpreted as R and an AST device result of S

Another representation of the concept:

$$\frac{N_{VMD} \times 100}{N_{RREF}}$$

where

N_{VMD} is the number of tests that result in a VMD;

N_{RREF} is the number of resistant bacterial isolates as determined by the reference method (ISO 20776-1)

NOTE The overall VMD is expressed as a percentage.

3.7
evaluation plan
description of a planned performance evaluation

3.8
evaluation report
description of and conclusions from a performance evaluation

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3.9 Clinical isolates

3.9.1
fresh isolate
isolate recovered from a clinical sample within the previous seven days that has not been frozen or subcultured more than five times

3.9.2
recent isolate
isolate recovered from a clinical sample within the previous twelve months

3.9.3
stock isolate
isolate recovered from a clinical sample that has been retained, stored or obtained from a culture collection

NOTE 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 fresh clinical isolates used in the evaluation.

3.10
investigator
person responsible for the execution of the performance evaluation at a certain location

3.11
minimum inhibitory concentration
MIC
lowest concentration that, under defined *in vitro* conditions, prevents visible growth of bacteria within a defined period of time

NOTE The MIC is expressed in mg/l.

3.12
MIC test
test that is capable of determining an MIC covering a range of at least five consecutive doubling dilutions, and for which EA can be determined

3.13
on-scale MIC test result
result from a MIC test when there is growth in at least one but not all concentrations tested

3.14
reference method
reference method described in ISO 20776-1

3.15
zone diameter
diameter (in mm) of the zone of growth inhibition around a disk containing an antimicrobial agent in an agar diffusion test

4 General requirements for a performance evaluation

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. The coordinator shall assess and document breakpoint criteria used and indicate which performance claims are met.

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5 Test methods

5.1 Overview

An evaluation conducted by a manufacturer shall consist of accuracy, reproducibility and quality control testing performed in at least three different laboratories, of which a maximum of one may be the manufacturer's laboratory. Testing shall be conducted using both the test device and the reference method.

5.2 Methods

5.2.1 Strain selection

An evaluation protocol should incorporate at least 300 clinical isolates relevant to an antimicrobial agent. Only one isolate per species per patient shall be included. The collection should include fresh and/or recent isolates from as many genera and species as feasible within the intended use of the device. It should include as many unrelated strains representing different degrees of susceptibility to the antimicrobial agents as possible. If a device is intended for testing a single genus or species, at least 100 clinical isolates should be studied. Stock isolates may be used to supplement the fresh or recent clinical isolates in order to provide resistant strains with different resistance mechanisms. A set of strains shall be defined to assess intra- and inter-laboratory reproducibility of the AST device. The quality control strain collection shall, as a minimum, include strains defined in the AST device package insert and any other strain(s) needed to provide on-scale results.

5.2.2 Isolate testing protocol

Isolate testing for the device shall be according to the manufacturer's instructions for use. Comparison of the test device result is made to the MICs of the reference method and the appropriately generated interpretations.

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

5.2.3 Inoculum preparation

The reference method and the test device shall be set up on the same day from the same inoculum source. The standardization of the inoculum for the test device shall be according to the manufacturer's instructions for use.

5.2.4 Reproducibility testing of test device

Triplicate testing of a minimum of ten strains (whenever possible including those with on-scale MIC test results for the antimicrobial agent being tested) shall be carried out on at least three days at each site where the test device is under evaluation. The number of on-scale isolates should be indicated in the final report. For breakpoint devices (excluding disk diffusion), the selection of strains should not include strains that are within one dilution of the breakpoint.

5.2.5 Quality control (QC) of the reference method

The quality control strains shall be tested every day testing is performed.

If QC results for any antimicrobial agent/bacterium combination are out of range on the reference method and the antimicrobial agent has only one on-scale QC organism, all testing for that day shall be repeated for that antimicrobial agent with both the reference method and test device.

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For antimicrobial agents with two or more on-scale QC organisms, the following apply.

a) If QC results for one antimicrobial agent/bacterium combination are out of range on the reference method, whilst the other QC strain(s) is (are) within the expected range, the test results for that antimicrobial agent/bacterium combination for that day may be acceptable if the QC results are within the expected range on the next testing day.

b) If the QC result for any antimicrobial agent/bacterium combination is out of range on the reference method for two successive days, both day's results shall be repeated for that antimicrobial agent with both the reference method and test device.

c) If QC results for two or more on-scale QC organisms are out of range on the reference method for any antimicrobial agent/bacterium combination on one day, all testing for that day shall be repeated for that antimicrobial agent with both the reference method and test device.

5.2.6 Results

For MIC devices, overall EA shall be calculated. For all methods, overall CA shall be calculated and presented according to the appropriate interpretive breakpoints claimed by the manufacturer.

5.2.7 Discrepancy resolution testing

Discrepancy resolution testing of the VMD and MD may be performed. If there is reasonable evidence of a technical error (e.g. mixed culture was tested, wrong incubation conditions), both the reference and the test method shall be repeated individually and the repeat results generated shall replace the original results.

If there is no obvious indication of a technical error, the discrepancy may be resolved by a one time triplicate testing of the reference method using separate bacterial inoculum suspensions. The categorical mode of the triplicate results for the reference method may replace the original result for purposes of determining the error rate if certain criteria are met. First of all, if there is an intermediate breakpoint, at least two of the three results shall give the same category agreement according to Table 1.

Table 1 — Combination of results of three repeat tests that allow an acceptable consensus result to be established

Repeat reference result N_1	Repeat reference result N_2	Repeat reference result N_3	Acceptable consensus result
S	S	S	S
S	S	I	S
S	I	I	I
I	I	I	I
R	I	I	I
R	R	I	R
R	R	R	R

Results obtained from triplicate testing of the reference method that do not accord with those shown in Table 1 are unacceptable and the results derived from that isolate shall be removed from the overall analysis.

For antimicrobial agents not having an intermediate category, i.e. S/R, two of the three repeat values represent the consensus result. All of the three repeat reference MIC values shall be within a three doubling dilution interval of each other.

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