EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Standard Operating Procedure

Procedure for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents

EUCAST SOP 9.3

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Foreword

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002.

The current remit of EUCAST is to harmonise clinical breakpoints for existing antimicrobial agents in Europe, to determine clinical breakpoints for new agents, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

Information on EUCAST, EUCAST breakpoints and all documents are freely available on the EUCAST website at www.eucast.org.

Citation of EUCAST documents

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EUCAST documents published on the EUCAST website should be cited in the following way: European Committee on Antimicrobial Susceptibility Testing. Name of document, EUCAST version number, year. Website address.

This SOP should be cited as: “European Committee on Antimicrobial Susceptibility Testing. Procedure for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents, EUCAST SOP 9.3, 2022. https://www.eucast.org/eucastsops/.

Abbreviations

AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESCMID	European Society for Clinical Microbiology and Infectious Diseases
EDL	EUCAST Development Laboratory
ISO	International Organization for Standardisation
MH	Mueller-Hinton
MIC	Minimum inhibitory concentration
beta-NAD	beta-Nicotinamide adenine dinucleotide
QC	Quality control
SOP	Standard Operating Procedure

Contents

Section		Page
	Foreword	3
	Citation of EUCAST documents	3
	Abbreviations	4
	Contents	5
1	Scope	6
2	Introduction	6
3	Evaluation of disks with the selected content (potency)	7
4	Defining relevant QC strains	8
5	Establishing MIC and zone diameter targets and acceptable ranges for QC strains	8
6	Establishing zone diameter breakpoints for new antimicrobial agents	10
7	Checklist for manufacturers	12

1 Scope

1.1 This SOP describes the necessary interaction between EUCAST, the EUCAST Development Laboratory (EDL) and when required, pharmaceutical companies to define the correlation between MIC and zone diameters to allow EUCAST to determine zone diameter breakpoints. The SOP also describes how quality control (QC) criteria for new antimicrobial agents are developed.

This relates specifically to:

- Evaluation of disks of the selected content (potency)
- Identifying and defining relevant QC strains
- Establishing targets and acceptable ranges for QC strains
- Determining the correlation between MIC values and inhibition zone diameters to define zone diameter breakpoints.

The selection of the optimal disk content (potency) is described in “European Committee on Antimicrobial Susceptibility Testing. Procedure for optimizing disk contents (potencies) for disk diffusion testing of antimicrobial agents using harmonized CLSI and EUCAST criteria. EUCAST SOP 11.x. https://www.eucast.org/eucastsops/.

2 Introduction

2.1 Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing (AST) and remains one of the most versatile and widely used AST methods in routine clinical laboratories.

Disk diffusion methods are influenced by the quality and variability in materials (i.e. antimicrobial disks and testing media) from different manufacturers and between batches from the same manufacturer. Some of this variability must be accepted to allow more than one manufacturer on the market. At the same time, it needs to be checked and controlled.

The selection of the optimal disk content (potency) is crucial to the development of a reproducible and reliable disk diffusion test, see “European Committee on Antimicrobial Susceptibility Testing. Procedure for optimizing disk contents (potencies) for disk diffusion testing of antimicrobial agents using harmonized CLSI and EUCAST criteria. EUCAST SOP 11.x https://www.eucast.org/eucastsops/.

This SOP describes the EUCAST requirements for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents after selecting the optimal disk content (potency).

3	Evaluation of disks with the selected content (potency)
3.1	**Evaluation of commercially produced disks** Once the optimal disk content (potency) is selected (in accordance with the joint procedure with CLSI, see SOP 11.x), commercially produced disks from at least two manufacturers should be investigated to ensure that (i) disks from a single cartridge exhibit the same content (potency) and (ii) that disks from different manufacturers are equally potent (produce near identical inhibition zone diameters). Following an agreement between the pharmaceutical company and the EDL, this can be performed by the EDL or another laboratory. Irrespective of which, the data and the data analysis need to be made available to and approved by EUCAST before acceptance in EUCAST breakpoint and QC tables.
3.2	**Reproducibility within a batch of disks** Zone diameters produced by disks from a single batch (≥ 20 disks from one vial/cartridge) with all other parameters (inoculum suspension, medium, incubation and reading) as constant as possible, should be within ±1 mm of the mean value.
3.3	**Reproducibility of disks between manufacturers** Disks from the multiple manufacturers should be tested in parallel (on the same agar plate) for all target species, and for relevant QC strains to ensure that the disks produce zone diameters within 1 mm when tested on the same agar plate. The most important target species should be included when evaluating organism groups, e.g. *Escherichia coli* and *Klebsiella pneumoniae* for *Enterobacterales*. One wild-type isolate and one non-wild type isolate (i.e. an isolate with elevated MICs and/or relevant acquired resistance mechanisms for the agent in question) per target organism should be included in the study. The non-wild type isolate must produce an inhibition zone (>6 mm). If no non-wild type isolates are available, testing is performed with two wild-type isolates per species having reproducibly different MICs. Testing should be performed on agar (Mueller-Hinton, Mueller-Hinton-F, Fastidious Anaerobe) from at least two manufacturers in parallel. The Mueller-Hinton media used must meet the requirements in ISO/TS 16782:2016 and the QC criteria published by EUCAST for standard QC strains.
3.4	Manufacturers, where disks do not meet potency criteria (same potency for all disks in each vial, same potency as already accepted disks, potency which meets QC

	criteria), are alerted to the identified problems and if there is no amendment, a warning against the disks in question may be posted on the EUCAST website.

4	Defining relevant QC strains
4.1	The choice of QC strains is based on which target species have been identified as targets for clinical use. In principle, criteria are developed for strains belonging to species against which the drug has clinically useful activity and which the manufacturer aims to include in the registration process. If the selected strain is already part of the existing EUCAST QC tables, the decision is normally simple and is taken together with the EDL.
4.2	Where it is important to detect or characterise a particular resistance mechanism, it may be necessary to develop specific criteria for supplementary strains displaying that resistance mechanism.
4.3	For disks consisting of combinations of agents (agent plus agent, agent plus inhibitor without antimicrobial activity, agent plus inhibitor with antimicrobial activity), multiple QC strains are often needed to control both components. A decision on the selection of QC strains for these disks is taken together with the EDL

5	Establishing MIC and zone diameter targets and acceptable ranges for QC strains
5.1	**Establishment of new MIC targets and ranges (for new agents or for existing agents when these have not previously been determined)** *1. Initial two-site study* The initial two-site study to establish a tentative QC range includes testing of each appropriate QC strain according to EUCAST recommendations and the following criteria: Each strain is tested on five separate days with three replicates (three individual inoculum suspensions) per day with broth media from at least two manufacturers. An appropriate control agent, preferably representing an antimicrobial agent belonging to the same group as the test substance, is included in all tests. This is to ensure that materials and procedures perform as expected and within the specified quality control ranges. It is also to

	determine whether the new agent exhibits a similar degree of variability in test systems as the related control agent. • Testing at the two sites is performed with the same lots of MIC panels and media. The range of concentrations tested must be such that all MIC results are within the tested range and truncation thus avoided. Both lyophilised and frozen panels are acceptable. *2. Validation involving additional laboratories* After the initial two-site study, a tentative QC target and range are validated by four to six additional laboratories (EUCAST Network Laboratories) with 10 replicates per site, each site using the media which are used locally. A control agent is included in all tests to ensure that materials and procedures perform as expected.
5.2	**Validation of existing quality control MIC ranges for fastidious organisms** For fastidious organisms, the MIC ranges in CLSI QC tables were determined using media with other supplements than those used by EUCAST. In the EUCAST method, the broth is supplemented with lysed horse blood and beta-NAD for fastidious organisms, including streptococci. For *Haemophilus influenzae,* there are no recommendations in the ISO method and the CLSI method adds different supplements to the MH broth. For anaerobic bacteria, EUCAST criteria are based on the use of Fastidious Anaerobe Agar (FAA), available from several manufacturers, and used without additives beyond the 5% mechanically defibrinated horse blood. Disk diffusion results are calibrated against MICs-values obtained with agar dilution on FAA. Results obtained with 10 repeated tests performed by broth microdilution with EUCAST media are compared with control ranges published by CLSI. If the MIC values in these tests are within the published range and are close to the middle of the range, the CLSI control range is adopted as tentative, and the midpoint of the range defined as the target. If not, the MIC target and ranges are determined as described above for new agents.
5.3	**Establishment of new zone diameter targets and ranges (for new agents or when these are unavailable for existing agents)** *1. Initial two-site study* The initial two-site study to establish a tentative QC range includes testing of each appropriate QC strain according to EUCAST recommendations and the following criteria:

	• Each strain is tested on five separate days with three replicates (three individual inoculum suspensions) per day on media (MH or MH-F plates as appropriate) from at least three manufacturers and with disks from at least two manufacturers. An appropriate control agent, preferably representing an antimicrobial agent belonging to the same group as the test substance, is included in all tests. • Testing at the two sites is performed on the same lots of media and disks. • Both in-house prepared agar plates and commercial agar plates are included. The Mueller-Hinton media used must meet the requirements in ISO/TS 16782:2016 and the QC criteria published by EUCAST for standard QC strains. *2. Validation involving additional laboratories* After the initial two-site study, the tentative QC range is validated by four or more additional laboratories (EUCAST Network Laboratories) with 10 repetitions per site using local media. Disks from at least two manufacturers are tested at each site. Disks of at least one control agent are included in all tests to ensure that materials and procedures perform within the specified quality control ranges and to determine whether the new agent exhibits the same degree of variability in the test systems as the control agent.

6	**Establishing zone diameter breakpoints for new antimicrobial agents**
6.1	EUCAST zone diameter breakpoints for new antimicrobial agents are established by the EUCAST Steering Committee based on the preparatory work and data performed or approved by the EDL.
6.2	**Correlation between MIC values and inhibition zone diameters** Once the disk content (potency) is established (see SOP 11) and the antimicrobial disks have been checked for reproducibility within batches and between manufacturers (see Section 3), disk diffusion (EUCAST methodology) and broth microdilution (ISO standard 20776-1, 2019, with EUCAST supplements for fastidious organisms, when relevant) are performed in parallel. At least 100 isolates of each relevant species are included. For groups of organisms, such as *Enterobacterales*, at least 200 isolates are included. Appropriate organisms, depending on the agent, would include: • *Escherichia coli* • Other *Enterobacterales* • *Pseudomonas* spp. • *Stenotrophomonas maltophilia*

- *Acinetobacter* spp.
- *Staphylococcus aureus*
- Coagulase-negative staphylococci
- *Enterococcus* spp.
- *Streptococcus* groups A, B, C and G
- *Streptococcus pneumoniae*
- Viridans group streptococci
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Pasteurella multocida*
- *Listeria monocytogenes*
- *Campylobacter jejuni* and *coli*
- *Corynebacterium* spp.
- *Aerococcus sanguinicola* and *urinae*
- *Kingella kingae*
- *Aeromonas* spp.
- *Achromobacter xylosoxidans*
- *Vibrio* spp.
- *Burkholderia pseudomallei*
- *Bacillus* spp.
- *Bacteroides* spp.
- *Prevotella* spp.
- *Fusobacterium necrophorum*
- *Clostridium perfringens*
- *Clostridioles difficile*
- *Cutibacterium acnes*

Each organism (or group of organisms) must include both wild-type and non-wild type isolates. At least 50% of the isolates should be devoid of resistance mechanisms (i.e. wild-type isolates) and these isolates must be identifiable in records. As many isolates as possible with MIC values close to the EUCAST clinical MIC breakpoint should be included. Highly resistant isolates with off-scale MIC and zone diameter values should be avoided when possible.

Disk diffusion testing must include Mueller-Hinton agar (and for anaerobic bacteria, FAA) and disks from at least two manufacturers. The Mueller-Hinton media used must meet the requirements in ISO/TS 16782:2016 and the QC criteria published by EUCAST for standard QC strains. Disks of at least one appropriate control agent, preferably representing an antimicrobial agent belonging to the same group as the test substance, must be included in all tests to ensure that materials and procedures perform within the specified quality control ranges, and to determine whether the new agent exhibits the same degree of variability in the test systems as the control agent. Relevant QC strains are tested in parallel (at least 10 consecutive tests) on both agars and with disks from both manufacturers.

6.3	**Zone diameter distributions of consecutive clinical isolates** As part of the establishment of zone diameter breakpoints, distributions of consecutive clinical isolates of target species should be produced. These should consist of 50-100 isolates per species from each of at least four laboratories (EUCAST Network Laboratories). Testing is performed on local media at each laboratory using disks provided by the EDL. At least one control agent with established breakpoints and QC criteria should be included in all tests.
6.4	**Data analysis and establishment of zone diameter breakpoints** All results are evaluated by the EUCAST staff and discussed with the pharmaceutical company. The final decision on zone diameter breakpoints is made by the EUCAST Steering Committee together with the EDL. The zone diameter breakpoints are tentative for one year. After one year the tentative breakpoints become established breakpoints unless there are issues which require further consideration. Normally, the process for determination of QC targets and ranges and zone diameter breakpoints do not require the company to present data to the EUCAST Steering Committee.

7	**Checklist for manufacturers**
7.1	It is recommended that manufacturers contact EUCAST at an early stage to discuss the development of QC criteria and the establishment of zone diameter breakpoints for new agents.
7.2	For EUCAST to establish QC targets and ranges and zone diameter breakpoints, it is necessary for manufacturers to provide or generate information on: • Intended target organisms (species), i.e. species that are likely clinical important targets for the agent. • Necessary special testing conditions. If EUCAST standard media (MH for non-fastidious organisms, MH-F for fastidious organisms or FAA for anaerobic bacteria) are not likely to be suitable, the company should consult with the EDL prior to any further development. • Whether QC criteria and zone diameter breakpoints have been developed for another organisation.