Standard Operating Procedure

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

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</thead>
<tbody>
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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), the European Centre for Disease Prevention and Control (ECDC), 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 http://www.EUCAST.org.

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>Antimicrobial susceptibility testing</td>
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<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
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<td>ESCMID</td>
<td>European Society for Clinical Microbiology and Infectious Diseases</td>
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<td>EDL</td>
<td>EUCAST Development Laboratory</td>
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<td>ISO</td>
<td>International Organization for Standardization</td>
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<td>MH</td>
<td>Mueller-Hinton</td>
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<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<tr>
<td>β-NAD</td>
<td>β-Nicotinamide adenine dinucleotide</td>
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<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
</tbody>
</table>
Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>3</td>
</tr>
<tr>
<td>Citation of EUCAST documents</td>
<td>3</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>4</td>
</tr>
<tr>
<td>Contents</td>
<td>5</td>
</tr>
<tr>
<td>1 Scope</td>
<td>6</td>
</tr>
<tr>
<td>2 Introduction</td>
<td>6</td>
</tr>
<tr>
<td>3 Determination of appropriate disk potency and evaluation of disks</td>
<td>6</td>
</tr>
<tr>
<td>4 Defining relevant QC strains</td>
<td>9</td>
</tr>
<tr>
<td>5 Establishing MIC and zone diameter targets and acceptable ranges for QC strains</td>
<td>9</td>
</tr>
<tr>
<td>6 Establishing zone diameter breakpoints for new antimicrobial agents</td>
<td>11</td>
</tr>
<tr>
<td>7 Checklist for manufacturers</td>
<td>13</td>
</tr>
</tbody>
</table>
1 **Scope**

1.1 This SOP describes the necessary interaction between EUCAST, the EUCAST Development Laboratory (EDL) and pharmaceutical companies to determine zone diameter breakpoints correlating with clinical MIC breakpoints and quality control (QC) criteria for new antimicrobial agents.

This relates specifically to:

- Determining disk potency and evaluation of disks
- Defining relevant QC strains
- Establishing targets and acceptable ranges for QC strains

Developing correlations between MIC values and inhibition zone diameters to define zone diameter breakpoints.

2 **Introduction**

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

Disk diffusion methods are influenced by the variability in materials from different manufacturers and between batches from the same manufacturer. This variability is in the disk potency and in the Mueller-Hinton agar/broth. 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.

This SOP describes the EUCAST requirements for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents.

3 **Determination of appropriate disk potency and evaluation of disks**

3.1 Setting disk potency

Disk potency is discussed with the pharmaceutical company and the disk manufacturers and normally requires some preliminary work by the disk manufacturers in collaboration with the EUCAST Development Laboratory. The preliminary work does not need to be performed at the EDL, but EUCAST should be involved in the process and the analysis. The preliminary work should ensure that wild-type isolates (isolates without...
phenotypically detectable resistance mechanisms) of all relevant species should exhibit zone diameter distributions from 15 mm to 35 mm. Smaller zone sizes than 15 mm should normally be avoided since the test will not safely discriminate between low and high level resistance. Larger zone sizes than 35 mm for target organisms should be avoided since this will increase the risk of interference between antibiotics on the disk diffusion plate reduce the number of disks that can be used on a plate. See figures 1 and 2 respectively for examples of an appropriate disk potency and a disk that is too potent. In general, the disk potency should be as low as possible but there should be room to distinguish between low-level and high-level resistance when appropriate

![Graph](image_url)

**Figure 1.** Example of inhibition zone distribution with an appropriate disk potency.

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**Procedure for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents**
3.2 Reproducibility within a batch of disks

As part of the evaluation of disk potency, it should be established that all disks from a single cartridge exhibit the same potency. Zone diameters produced by disks from one single batch (≥20 disks) with all other parameters (inoculum suspension, media, incubation and reading) as constant as possible should be within ±1 mm of the mean. The EDL will perform this check as part of the procedure for deciding on disk potency.

3.3 Multiple disk potencies

On rare occasions, EUCAST has recommended two different disk potencies to accommodate both very sensitive and less sensitive organisms. For example, the EUCAST list of disks includes ampicillin 2 µg and 10 µg disks for different organism groups.

Figure 2. Example of inhibition zone distributions with a too potent disk.
### 3.4 Decisions on disk potency

The final decision concerning the disk potency for the EUCAST disk diffusion test is made by the EDL or, if contentious, by the EUCAST Steering Committee.

### 3.5 Reproducibility of disks between manufacturers

After the appropriate disk potency has been set, the company should obtain disks from at least two, and preferably more, disk manufacturers. This is to ensure that the disk potency is reproducible between manufacturers and that generic QC criteria are developed.

The EDL will compare performance of disks against all target organisms and assist in planning necessary QC studies. Disks from the multiple manufacturers should be tested in parallel for all target organisms, and for relevant QC strains. Both wild-type isolates and isolates with elevated MICs and/or relevant resistance mechanisms for the agent in question must be included in the study. A total of 5-10 isolates per organism or group of organisms must be tested.

### 4 Defining relevant QC strains

#### 4.1

The target species, as reported by the company, determines for which QC strains control criteria will be developed. 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. The EDL will take the final decision.

#### 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.

### 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 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.
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 (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 within the specified quality control ranges, and to determine whether the new agent exhibits a similar degree of variability in the 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. Both lyophilised and frozen panels are accepted.

2. Validation involving additional laboratories

After the initial two-site study, the tentative QC range is validated by testing at 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 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.

5.2 Validation of existing quality control MIC ranges for fastidious organisms

For fastidious organisms the MIC ranges in QC tables from ISO (ISO standard 20776-1, 2006) and CLSI were determined using broth medium with other supplements than those used by EUCAST. In the EUCAST method, the broth is supplemented with β-NAD for fastidious organisms, including streptococci. For *Haemophilus influenzae* there are no recommendations in the ISO method and the CLSI method adds a different supplement to the MH broth.

Results obtained with 10 repeated tests performed by broth microdilution with EUCAST media are compared with control ranges published by ISO or CLSI. If the MIC values in these tests are within the published range and are close to the middle of the range, the ISO or 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-Fplates as appropriate) from at least three manufacturers and with disks from at least two manufacturers. An appropriate control disk, 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

2. Validation involving additional laboratories

After the initial two-site study, the tentative QC range is validated by testing at 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 and based on the preparatory work performed by the EDL. This is performed following an agreement between the pharmaceutical company and the EDL.

6.2 Correlation between MIC values and inhibition zone diameters

Once the disk potency is established and the antimicrobial disks have been checked for reproducibility within batches and between manufacturers (see Section I), inhibition zone diameters are determined for isolates with known MIC values, determined using the ISO standard 20776-1, 2006, with EUCAST supplements for fastidious organisms, when relevant). At least 100 isolates of each relevant species are included.

Appropriate organisms, depending on the agent, would include:

- *Escherichia coli*
- Other Enterobacteriaceae
- *Pseudomonas* spp.
Each organism (or group of organisms) must include both wild-type and resistant isolates. At least 50% of the isolates should be devoid of resistance mechanisms. 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 disks from at least two manufacturers. Disks of at least one appropriate control agent, 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.

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 development. Normally, the process for determination of QC breakpoints.
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).
- Intended disk potency (with results of preliminary work done to support any specific disk potency and including work performed on alternative disk potencies). Note that EUCAST will not automatically accept QC criteria and zone diameter breakpoints developed by other groups.
- Performance of tests with disks from more than one disk manufacturer.
- Necessary special testing conditions
- Whether QC criteria and disk diffusion breakpoints have been developed for another organisation