CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

CLSI M23S™

Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria

CLSI M23S describes the necessary technical steps for establishing the optimal disk content (potency) for single antimicrobial agents without the addition of enhancing or inhibiting substances.

A CLSI supplement for global application.

CLSI M23S-Ed2
November 2024
Replaces CLSI M23S-Ed1

Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria

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Abstract

Clinical and Laboratory Standards Institute M23S—*Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria* describes the necessary technical steps for establishing the optimal disk content (potency) for single antimicrobial agents without the addition of enhancing or inhibiting substances.

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NOTE: The content in CLSI M23S is identical to the content 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 11.1, 2024. http://www.eucast.org."

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
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Contents

Foreword

The disk diffusion antimicrobial susceptibility test has been widely used around the world for decades and was first standardized in 1966.[1] In the 1970s, CLSI (then the National Committee for Clinical Laboratory Standards) published additional guidance for disk diffusion testing. In Europe, different variants of the disk diffusion method were used in different countries until 2009, when the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provided a standardized disk diffusion method calibrated to the harmonized European minimal inhibitory concentration breakpoints. The disk diffusion test is based on incorporating a standard amount of an antimicrobial agent into a filter paper disk. Because it is relatively easy to perform and uses standard microbiology laboratory equipment, the disk diffusion test is used in many types of laboratories, including those in low-resource settings.

The disk content (potency) recommended for new antimicrobial agents has sometimes varied among organizations that set criteria (eg, breakpoints) for interpreting results of disk diffusion testing. Subsequently, pharmaceutical manufacturers have performed testing with two different disk contents (potencies) for generating data to present to breakpoint-setting organizations. This burdensome situation was caused in part by a lack of harmonized recommendations for selecting optimal disk contents (potencies). To correct this issue and improve efficiency for pharmaceutical manufacturers, disk manufacturers, researchers, and other organizations, CLSI and EUCAST initiated a joint venture to develop standardized recommendations for disk content (potency) selection. Their recommendations are presented in CLSI M23S.

Contact information: clsi.org/m23-supplement-question

CLSI
www.clsi.org

EUCAST
www.EUCAST.org

Overview of Changes

CLSI M23S-Ed2 replaces CLSI M23S-Ed1, published in 2020. Several changes were made in this edition, including:

- Adding suggested actions to Subchapter 1.2 for a company seeking approval from CLSI and/or EUCAST for a disk for which the content (potency) was not developed in collaboration with the joint CLSI-EUCAST working group
- Adding recommendations to Subchapter 2.2 for evaluating ease of reading and weight of growth
- Expanding recommendations in Subchapter 2.5 for developing the optimal disk content (potency) for combination agents

NOTE: The content of CLSI M23S is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

disk content **disk diffusion** **disk potency**

Chapter 1

Introduction

Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria

1 Introduction

1.1 Scope

CLSI M23S is intended for pharmaceutical manufacturers involved in the development of antimicrobial agents and tests to support evaluation of antimicrobial agent activity for testing of bacteria. It is also intended for manufacturers of antimicrobial disks and any independent laboratory that supports the development of these disks. CLSI M23S describes the process for selecting the optimal content (potency) of antimicrobial agent to be added to filter paper disks to obtain reliable results with the standardized disk diffusion test. It does not explain the steps needed to perform the standardized disk diffusion test, nor does it define the criteria (breakpoints) used to interpret zone diameters of inhibition into interpretive categories. These steps are described elsewhere (see CLSI M02[2] and CLSI M07[3]).[4,5] In some cases, the breakpoints defined by breakpoint-setting organizations for a single agent may differ even when the same disk content (potency) is used.

1.2 Background

The standard for antimicrobial susceptibility testing of rapidly growing aerobic bacteria is minimal inhibitory concentration (MIC) determination using broth microdilution according to international standards[6] or CLSI M07,[3] except for a few agents and/or organisms for which broth microdilution does not provide reliable results. For fastidious organisms, the basic methodology is the same, but CLSI (see CLSI M07[3]) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[7] recommend different media. Both CLSI (see CLSI M02[2]) and EUCAST[4] have developed standardized disk diffusion methods calibrated to match the results of reference MIC methodology (see CLSI M07[3])[7] based in part on a method originally described in 1966.[1] Optimal disk content (potency) selection for disk diffusion testing is critical for the development of an accurate and reproducible test. Disk contents (potencies) can be developed only once a reference MIC method has been established for the antimicrobial agent and organisms in question.

The CLSI and EUCAST disk diffusion methods are based on reproducible and reliable separation between isolates belonging to different interpretive categories as determined by reference MIC methodology. For each organism-agent combination, disk diffusion testing of clinical isolates should result in an on-scale zone diameter distribution that spans a 10- to 14-mm range for wild-type (WT) organisms (see examples in Appendix A). Populations with and without resistance mechanisms that are clearly distinguishable by MIC should also be clearly distinguishable by inhibition zone diameter. Determining the optimal disk content (potency) is integral to achieving this goal.

The CLSI and EUCAST disk diffusion methods are based on the same basic methodology, ie, Mueller-Hinton agar and an inoculum size equivalent to a 0.5 McFarland standard. At present, there are differences between CLSI and EUCAST in supplements for media for fastidious organisms and in disk contents (potencies) for some antimicrobial agents. Because having common disk content (potency) for both CLSI and EUCAST disk diffusion testing is an advantage to users of the disk diffusion methods, pharmaceutical companies, and disk manufacturers, the joint CLSI-EUCAST working group formed in 2017 has agreed on common criteria for development of optimal disk contents (potencies) to be incorporated into 6-mm filter paper disks for disk diffusion testing. These disks are endorsed by both CLSI and EUCAST. Pharmaceutical companies interested in

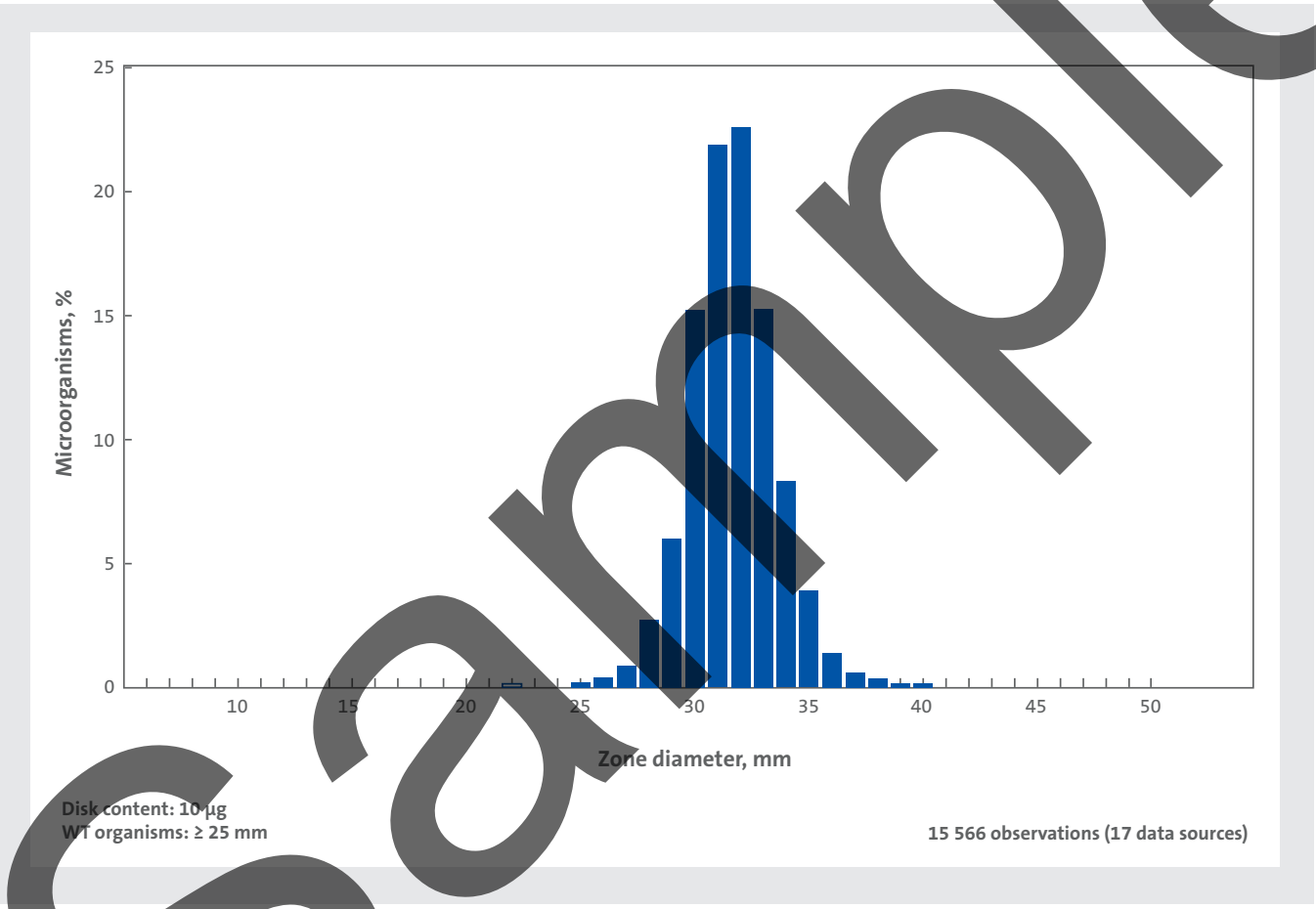
Appendix A. Examples of Zone Diameter Distributions With a Defined Wild-Type Distribution

Abbreviations for Appendix A

SD standard deviation

WT wild-type

On-scale zone diameter distributions (±2 SD) of wild-type (WT) organisms normally span 10 to 14 mm. **NOTE:** Zone diameter distributions with a defined WT distribution are represented by the blue bars in Figures A1 through A4. Non-wild-type distributions are represented by the white bars in Figures A1 through A4.



Abbreviation: WT, wild-type.

Figure A1. Zone Diameter Distribution for WT *Escherichia coli* and Meropenem.[1] Distributions include collated data from multiple sources, geographical areas, and time periods and cannot be used to infer rates of resistance. (European Committee on Antimicrobial Susceptibility Testing. MIC and zone diameter distributions and ECOFFs. Accessed 19 September 2024. https://www.eucast.org/mic_distributions_and_ecoffs/)

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