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M23S

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

This document 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.

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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 document 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 this document 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 11.0, 2020. http://www.eucast.org."

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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 content (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 selection. Their recommendations are presented in this document.

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EUCAST
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NOTE: The content of this document 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

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

Chapter 1: Introduction

This chapter includes:

- Document's scope and applicable exclusions
- Background information pertinent to the document's content
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the document
  - Abbreviations and acronyms used in the document

1.1 Scope

This document is intended for pharmaceutical manufacturers involved in the development of antimicrobial agents and tests to support evaluation of antimicrobial agent activity. It is also intended for manufacturers of antimicrobial disks and any independent laboratory that supports the development of these disks. This document 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 documents M02[2] and M07[3]).[4] 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[5] or CLSI document 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 document M02[2]) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[4] recommend different media. Both CLSI (see CLSI document M02[2]) and EUCAST[4] have developed standardized disk diffusion methods calibrated to match the results of reference MIC methodology (see CLSI document M07[3])[5,6] 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 only be developed 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 CLSI-EUCAST joint 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 having disk diffusion breakpoints published in CLSI and/or EUCAST tables should follow the procedure when developing disks for disk diffusion testing.

1.3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[7] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[8]

1.4 Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines. Table 1 is provided to clarify the intended interpretations of the following terms.

Table 1. Common Terms or Phrases With Intended Interpretations

Term or Phrase	Intended Interpretation
"Needs to" or "must"	Explains an action directly related to fulfilling a regulatory and/or accreditation requirement or is indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure
"Require"	Represents a statement that directly reflects a regulatory, accreditation, performance, product, or organizational requirement or a requirement or specification identified in an approved documentary standard
"Should"	Describes a recommendation provided in laboratory literature, a statement of good laboratory practice, or a suggestion for how to meet a requirement

- Options for obtaining reference MIC values for clinical isolates include:
  - Performing MIC testing in parallel with disk diffusion testing
  - Selecting isolates with previously established MIC values

- Isolates must be retested if the relationship between the MIC and zone diameter is not consistent with results from other similar isolates or not logical (ie, a low MIC and a small zone diameter or a high MIC and a large zone diameter). Retesting should be conducted using a single inoculum suspension for both reference MIC and disk diffusion methods in parallel. Three separate inoculum suspensions should be prepared to obtain triplicate results for each isolate.

- Disk diffusion must be performed using a Mueller-Hinton medium that meets the specifications in international standards[9] and the QC criteria published by CLSI (see CLSI document M100[10]) and EUCAST[11] for standard QC strains. To establish acceptable quality of the medium, results must be in range when testing QC strains and agents from similar and different antimicrobial classes. The numbers of QC strains and additional agents tested will vary depending on experience with particular lots of Mueller-Hinton medium used and the antimicrobial agent under investigation.

- For fastidious organisms, CLSI and EUCAST disk diffusion media must be tested in parallel.

- Testing can be performed on one or multiple days for clinical isolates.

- Relevant QC strains must be tested each day clinical isolates are tested and for a minimum of three separate days. The difference in zone diameter measurements obtained from testing a single QC strain or clinical isolate repetitively in one laboratory using the same lots of disks and media should not exceed 3 mm.

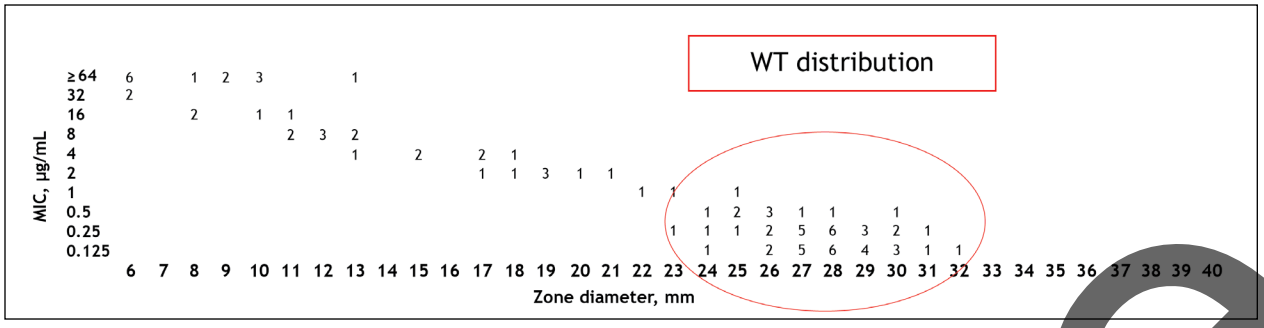
- An appropriate control agent (preferably an antimicrobial agent belonging to the same or similar class as the agent being evaluated) with CLSI (see CLSI document M100[10]) and/or EUCAST[11] published QC ranges must be included with disk diffusion testing of all isolates (clinical isolates and QC strains).

2.3 Phase 1: Initial Screening of a Series of Disk Contents (Potencies)

The aim of phase 1 is to screen up to 10 disks covering a wide range of contents (potencies) against a small number of isolates of the target species. From these results, the contents (potencies) of 2 to 4 disks will be selected for testing in phase 2 with a larger number of isolates.

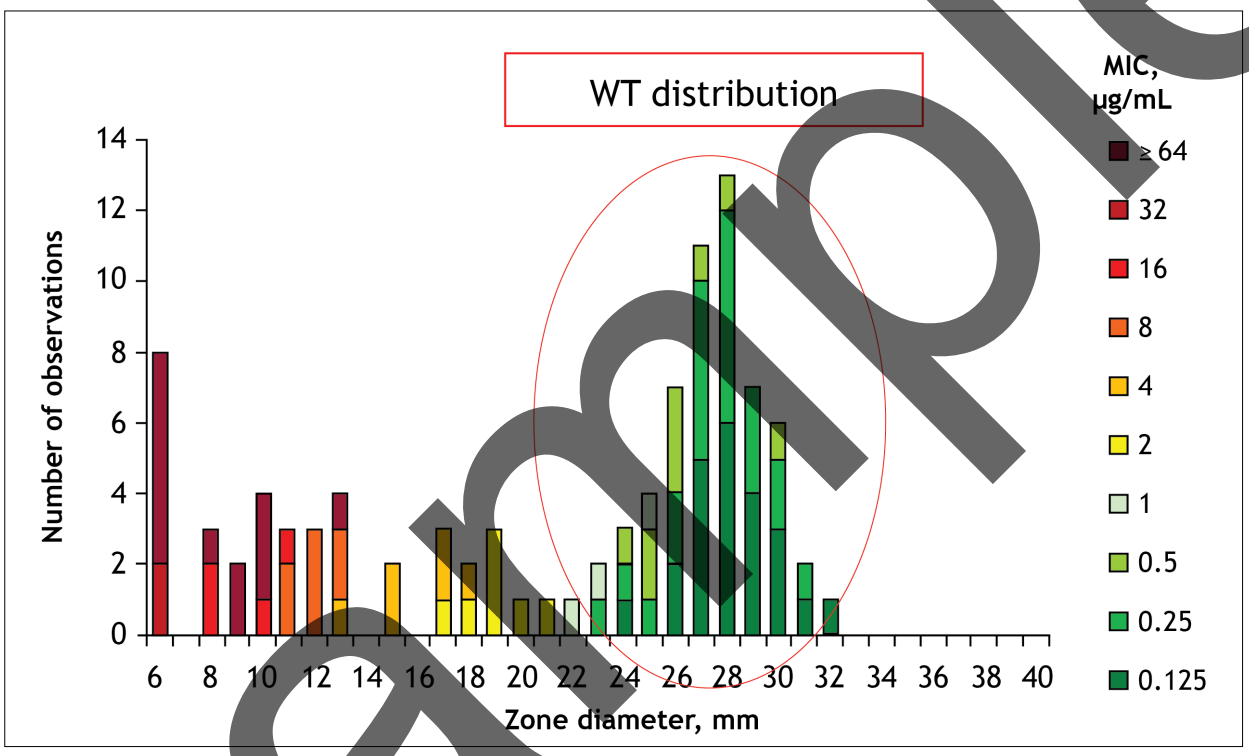
Normally, 10 different disks ranging from very low to very high (eg, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 µg) content (potency) are produced in small batches and tested according to standardized disk diffusion methodology against relevant species. The most important target species should be included when evaluating organism groups, eg, *Escherichia coli* and *Klebsiella pneumoniae* for Enterobacterales. For agents with broad-spectrum activity against a variety of organism groups (eg, gram-positive and gram-negative genera), it might be necessary to test disk contents (potencies) beyond the 0.1- to 100-µg range. The contents (potencies) of fewer than 10 disks can be evaluated, but the risk of having to repeat the study if none of the disk contents (potencies) tested performs reliably is increased.

- A disk content (potency) previously used for the antimicrobial class of the agent being evaluated (eg, 5 µg for fluoroquinolones, 30 µg for third-generation cephalosporins) should be included but should not be considered the optimal content (potency) by default.



Abbreviations: MIC, minimal inhibitory concentration; WT, wild-type.

Figure 1A. Zone Diameter Scattergram With Zone Diameters Plotted Against Minimal Inhibitory Concentration Values. Figures 1A and 1B represent the same dataset.



Abbreviations: MIC, minimal inhibitory concentration; WT, wild-type.

Figure 1B. Zone Diameter Histogram With MIC Values Represented by Colored Bars. Green corresponds to WT isolates. Yellow, orange, and red correspond to different minimal inhibitory concentrations for non-wild-type isolates. Figures 1A and 1B represent the same dataset.

2.4.2 Selection of Optimal Disk Content (Potency)

Optimal disk content (potency) is determined using the selection criteria listed in Subchapter 2.1 following visual review of the raw data and data displayed in scattergrams and histograms. WT and NWT populations, clearly distinguishable by MIC, should also be clearly distinguishable by inhibition zone diameter.

Related CLSI Reference Materials*

M02 **Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed., 2018.** This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

M07 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed., 2018.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M29 **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

M100 **Performance Standards for Antimicrobial Susceptibility Testing. 30th ed., 2020.** This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02, M07, and M11.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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