

Clinical laboratory testing and in vitro diagnostic test systems - Reference method for testing the in vitro activity of antimicrobial agents against yeast of fungi involved in infectious diseases (ISO 16256:2012)

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English Version

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agents against yeast of fungi involved in infectious diseases
(ISO 16256:2012)**

Essais de laboratoire clinique et systèmes de diagnostic in
vitro - Méthode de référence pour soumettre à essai
l'activité in vitro des agents antimicrobiens par rapport aux
levures impliquées dans les maladies infectieuses (ISO
16256:2012)

Labormedizinische Untersuchungen und In-vitro-
Diagnostika-Systeme - Referenzmethode zur Testung der
In-vitro-Aktivität von antimikrobiellen Substanzen gegen
Pilze, die Infektionskrankheiten verursachen (ISO
16256:2012)

This European Standard was approved by CEN on 30 November 2012.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Foreword

This document (EN ISO 16256:2012) has been prepared by Technical Committee ISO/TC 212 "Clinical laboratory testing and in vitro diagnostic test systems" in collaboration with Technical Committee CEN/TC 140 "In vitro diagnostic medical devices", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2013, and conflicting national standards shall be withdrawn at the latest by December 2015.

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Endorsement notice

The text of ISO 16256:2012 has been approved by CEN as a EN ISO 16256:2012 without any modification.

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Introduction

In vitro susceptibility tests are performed on microorganisms suspected of causing disease, particularly if the organism is thought to belong to a species that may exhibit acquired resistance to frequently used antimicrobial agents. The tests are also important in resistance surveillance, epidemiological studies of susceptibility and in comparisons of new and existing agents.

Dilution procedures are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and represent the reference method for antifungal susceptibility testing. MIC methods are used in resistance surveillance, comparative testing of new agents for research or registration purposes, to establish the susceptibility of organisms that give equivocal results in routine tests, for tests with organisms where routine tests may be unreliable and when a quantitative result is needed for clinical management. In dilution tests, microorganisms are tested for their ability to produce discernible growth on a series of agar plates (agar dilution) or in broth (broth dilution) containing serial dilutions of the antimicrobial agent.

The lowest concentration of an antimicrobial agent (in mg/l) that, under defined *in vitro* test conditions, reduces visible or optically measurable growth of a microorganism within a defined period of time is known as the MIC. The MIC is a guide for the clinician to the susceptibility of the organism to the antimicrobial agent and aids treatment decisions. Careful control and standardization is required for intra- and inter-laboratory reproducibility, as results may be influenced by the method used. It is generally accepted that broth MIC tests are reproducible to within one doubling dilution of the true end point (i.e. ± 1 well or tube in a doubling dilution series).

Broth dilution is a technique in which containers holding identical volumes of broth with antimicrobial agent solutions in incrementally (usually twofold) increasing concentrations are inoculated with a known number of microorganisms.

Broth microdilution denotes the performance of the broth dilution test in microdilution trays.

The reference methods described in this International Standard are intended for the testing of pure cultures of yeast fungi. The broth microdilution methods described in this part of this International Standard are essentially the same as those described by the Clinical and Laboratory Standards Institute (CLSI)^[1] and by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)^[2]. These methods have been shown to provide MICs of fluconazole that are essentially the same, if not identical up to 2 mg/l^[3]. Studies with various other antifungal agents are planned or under way. The laboratory that wishes to use this International Standard for conducting studies of newer antifungal agents, or as a reference method for comparison to MICs generated by a diagnostic device, should select which of the procedure options to use based upon the choice of MIC reading determined by visual inspection (CLSI method) or by use of a spectrophotometer (EUCAST method). In either case, the procedural details for that option are to be followed explicitly.

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WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard describes a method for testing the susceptibility to antifungal agents of yeasts, including *Candida* spp. and *Cryptococcus neoformans*, that cause infections. The reference method described here has not been used in studies of the yeast forms of dimorphic fungi, such as *B. dermatitidis* and/or *H. capsulatum* variety *capsulatum*. Moreover, testing filamentous fungi (moulds) introduces several additional problems in standardization not addressed by the current procedure. Reference methods for broth dilution antifungal susceptibility testing of filamentous fungi have been developed and are now available as CLSI document M38 and EUCAST document E.DEF 9.1[4][5][6][7][8].

This International Standard describes the broth microdilution reference method which can be implemented by either of two pathways. One pathway involves visual determination of MICs (CLSI method) [1]; the second pathway involves spectrophotometric determination of MICs (EUCAST method) [2]. The MIC reflects the activity of the drug under the described test conditions and can be interpreted for clinical management purposes by taking into account other factors, such as drug pharmacology or antifungal resistance mechanisms. MICs can be categorized as “susceptible” (S), “susceptible dose-dependent” (S-DD), “intermediate” (I), “non-susceptible” (NS) or “resistant” (R). In addition, MIC distributions can be used to define wild type or non-wild type fungal populations. Clinical interpretation of the MIC value is beyond the scope of this International Standard; interpretive category breakpoints specific to the CLSI- and EUCAST-derived methods can be found by consulting the latest interpretive tables provided by the organizations [2][9]. It is advisable to compare routine susceptibility testing methods or diagnostic test devices with this reference method in order to ensure comparable and reliable results for validation or registration purposes.

2 Terms and definitions

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

2.1

antifungal agent

substance of biological, semi-synthetic or synthetic origin that inhibits the growth of or kills fungi, and is thus of potential use in the treatment of infections

NOTE Disinfectants, antiseptics and preservatives are not included in this definition.