 10.1111/j.1469-0691.2007.01935.x

# EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)*

**Keywords** Antifungal agents, EUCAST, fermentative yeasts, MICs, susceptibility testing method

*Clin Microbiol Infect* 2008; **14:** 398–405

## INTRODUCTION

Antifungal susceptibility tests are performed on fungi that cause disease, especially if they belong to a species exhibiting resistance to commonly used antifungal agents. Antifungal susceptibility testing is also important for resistance surveillance, for epidemiological studies and for comparing the in-vitro activity of new and existing agents.

Dilution methods are used to establish the MICs of antimicrobial agents. These are the reference methods for antimicrobial susceptibility testing, and are used mainly to establish the activity of a new antifungal agent, to confirm the susceptibility of organisms that give equivocal results in routine tests, and to determine the susceptibility of fungi where routine dilution tests may be unreliable. Fungi are tested for their ability to produce visible growth in microdilution plate wells containing broth culture media and serial dilutions of the antifungal agents (broth microdilution). The MIC is defined as the lowest concentration (in mg/L) of an antifungal agent that inhibits the growth of a fungus. The MIC provides information concerning the susceptibility or resistance of an organism to the antifungal agent and can help in making correct treatment decisions.

The method described in this document is intended for testing the susceptibility of yeasts that cause clinically significant infections (primarily *Candida* spp.). The method encompasses only those yeasts that are able to ferment glucose. Thus, the susceptibility of non-fermentative yeasts, e.g., *Cryptococcus neoformans*, cannot be determined by the current procedure, and the method is not suitable for testing the yeast forms of dimorphic fungi.

## SCOPE

The standard method described in this document provides a valid method for testing the susceptibility of glucose-fermenting yeasts to antifungal agents by determination of the MIC. MICs indicate the activity of a given antifungal drug under the described test conditions, and can be used in making decisions concerning patient management after taking into account other factors, e.g., pharmacokinetics, pharmacodynamics and resistance mechanisms. The MIC also allows fungi to be categorised as 'susceptible' (S), 'intermediate' (I) or 'resistant' (R) to a drug. In addition, MIC distributions can be used to define wild-type or non-wild-type fungal populations.

This method is intended primarily to facilitate an acceptable degree of conformity, i.e., agreement within specified ranges among laboratories, in measuring the susceptibility of yeasts to antifungal agents. The method is designed to be easy to perform, rapid, economical, and suitable for reading by microdilution plate readers in order to allow direct transfer, storage and manipulation of the data by computer. The method is also intended to yield results that are concordant with those obtained using the procedure

Corresponding author and reprint requests: J. L. Rodriguez-Tudela, Mycology Reference Laboratory, National Center for Microbiology, Instituto de Salud Carlos III, Ctra. Majadahonda-Pozuelo Km.2, E-28220 Majadahonda, Spain
E-mail: jlrtudela@isciii.es

*J. L. Rodriguez-Tudela (Chairman, Spain), M. C. Arendrup (Denmark), F. Barchiesi (Italy), J. Bille (Switzerland), E. Chryssanthou (Sweden), M. Cuenca-Estrella (Spain), E. Dannaoui (France), D. W. Denning (UK), J. P. Donnelly (The Netherlands), F. Dromer (France), W. Fegeler (Germany), C. Lass-Flörl (Austria), C. Moore (UK), M. Richardson (Finland), P. Sandven (Norway), A. Velegraki (Greece) and P. Verweij (The Netherlands).

recommended by CLSI document M27 for antifungal susceptibility testing of yeasts [1].

## TERMS AND DEFINITIONS

### Antifungal agent

An antifungal agent is a substance of biological, semi-synthetic or synthetic origin that inhibits the growth of fungi or is lethal to them. Disinfectants, antiseptics and preservatives are not included in this definition.

### Potency

Potency is the antimicrobially active fraction of a test substance, determined in a bioassay against a reference powder of the same substance. The potency is expressed as mass fraction in mg/g, or as activity content in International Units (IU)/g, or as a volume fraction or mass fraction in per cent, or as an amount-of-substance concentration (mass fraction) in mol/L of the ingredients in the test substance.

### Concentration

Concentration is the amount of an antimicrobial agent in a defined volume of liquid. The concentration is expressed in SI units as mg/L. Although mg/L is equivalent to μg/mL, the use of the latter is not recommended.

### Stock solution

A stock solution is an initial solution used for additional dilutions.

## MIC

The MIC is the lowest concentration of a substance that inhibits the growth of fungi within a defined period of time. The MIC is expressed in mg/L.

### Breakpoint

Breakpoints are specific MIC values that enable fungi to be assigned to the clinical categories 'susceptible', 'intermediate' and 'resistant'. Breakpoints can be altered according to changes in circumstances (e.g., changes in commonly used drug dosages).

*Susceptible (S).* A fungal strain inhibited *in vitro* by a concentration of an antifungal agent that is associated with a high likelihood of therapeutic success. Fungi are categorised as susceptible by applying the appropriate breakpoints in a defined phenotypic test system.

*Intermediate (I).* A fungal strain inhibited *in vitro* by a concentration of an antifungal agent that is associated with a doubtful therapeutic effect. Fungal strains are categorised as intermediate by applying the appropriate breakpoints in a defined phenotypic test system. Intermediate susceptibility implies that an infection caused by the isolate can be treated effectively at body sites where the antifungal drug is physiologically concentrated or when a high dosage of drug can be used. This class also includes a 'buffer zone', to prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

*Resistant (R).* A fungal strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure. Fungal strains are categorised as resistant by applying the appropriate breakpoints in a defined phenotypic test system.

### Wild-type

The term 'Wild-type' refers to an isolate without acquired resistance mechanisms to the antifungal agent.

### Control strain

The term 'Control strain' refers to a catalogued, characterised strain with stable, defined antifungal susceptibility phenotypes and/or genotypes. Such strains are obtainable from culture collections and are used for quality control purposes.

### Broth dilution

Broth dilution is a susceptibility testing technique in which serial dilutions (usually two-fold) of an antifungal agent are made in a liquid medium that is inoculated with a standardised number of organisms and incubated for a prescribed period. The objective of this method is the determination of the MIC.