ENGLISH

EU AFST

Panel for Antifungal Susceptibility Testing with Broth Microdilution Method, according to ISO 16256

INTENDED PURPOSE

EU AFST is a panel for quantitative determination of the minimum inhibitory concentration (MIC) against yeasts.

DESCRIPTION

EU AFST is a panel containing eight different antifungal agents in different concentrations.
The device is used for evaluation of antifungal susceptibility test of yeasts.

Amphotericin B (AMB) concentration range
0.004 µg/mL to 2 µg/mL;
Fluconazole (FLU) concentration range
0.032 µg/mL to 32 µg/mL;
Itraconazole (ITC) concentration range
0.004 µg/mL to 4 µg/mL;
Voriconazole (VO) concentration range
0.004 µg/mL to 4 µg/mL;
Posaconazole (POS) concentration range
0.004 µg/mL to 4 µg/mL;
Anidulafungin (AND) concentration range
0.004 µg/mL to 4 µg/mL;
Micafungin (MYC) concentration range
0.004 µg/mL to 4 µg/mL;
Flucytosine (AFY) concentration range
0.016 µg/mL to 16 µg/mL;

KIT CONTENT

- 6 panels individually packed in plastic bag
- Instructions Sheet and Test Results Form

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as:

-loops - pipettes - physiological solution - swabs - culture media - incubator

-test tubes - 0.5 McFarland turbidity standard - quality control organisms -RPMI broth (Ref. 24461)

CONFIGURATION

		1	2	3	4	5	6	7	8	9	10	11	12
Amphotericin B	A	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	Negative control
Fluconazole	B	Positive control	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Itraconazole	C	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4
Voriconazole	D	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4
Posaconazole	E	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4
Anidulafungin	F	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4
Micafungin	G	Positive control	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4
Flucytosine	H	Positive control	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16

PRINCIPLE OF THE METHOD

All wells are inoculated with a standardized microbial suspension, with the exception of the well A12 that is inoculated with just RPMI broth.
After incubation the MIC result is read and interpreted.

SPECIMEN COLLECTION AND PREPARATION

EU AFST is not for use directly with clinical or other specimens. The microorganism to be tested must first be isolated on a nonselective culture medium. In case of mixed culture, selected colonies should be purified by subculturing. Differential media harboring chromogenic or fluorogenic substrates should not be used for the subculture. It is recommended that cultures be no more than 24 hours old unless additional incubation is required to achieve sufficient growth.

TEST PROCEDURE

1. Culture all yeasts in ambient air at 34 to 37°C on non-selective nutritive agar medium (e.g. Sabouraud's dextrose agar or potato dextrose agar) for 18-48 h before testing.
2. Take a panel from its envelope and leave it at room temperature for 10 min
3. Prepare the inoculum by suspending five distinct colonies, ≥1 mm diameter from 24 h cultures, in at least 3 mL of sterile distilled water. The suspension is homogenized for 15 seconds with a gyratory vortex mixer at approximately 2,000 rpm.
4. Adjust the cell density to the density of a 0.5 McFarland standard. This will give a yeast suspension of 1-5 x 10^6 CFU/mL.
5. Prepare a working suspension by a 1 in 20 dilution of the standardized suspension in RPMI broth.
6. Dispense 200 µL of the solution into each well, except the negative control (A12). For negative control, add 200 µL of RPMI broth.
7. Cover the panel with the lid provided and incubate at 35 ± 2°C for 24-48 hours in ambient air.

READING THE RESULTS

At the end of the incubation period, observe the growth in the wells and determine the MIC value.

The microdilution plates can be read using a microdilution plate reader (recommended wavelengths 530,405,450 or 490 nm) according to EUCAST Definitive Document E.Def 7.4, or visually, following by *Photographic Reading Guide.*

The growth-control well should be evaluated first. Make sure it is positive for growth. If not, check the viability of the colonies picked and repeat the test using a new panel and a microbial culture of recent growth.

Note the results on the Test Results Form (copy as many forms as necessary).

INTERPRETATION OF THE RESULTS

The MIC obtained should be interpreted according to current EUCAST criteria.

USER QUALITY CONTROL

QC of EU AFST is performed using the following reference strains:

Control strains	AMB (µg/mL)	FLU (µg/mL)	ITC (µg/mL)	VO (µg/mL)	POS (µg/mL)	AND (µg/mL)	MYC (µg/mL)	AFY (µg/mL)
Candida parapsilosis ATCC® 22019	0.125-1	0.5-2	0.03-0.125	0.016-0.06	0.016-0.06	0.25-1	0.5-2	0.125-0.5

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

WARNINGS AND PRECAUTIONS

1) **For research use only (RUO).**
2) **For laboratory professional use only.**
3) **The panel is for single use only and should not be reused.**
4) Operators must be trained and have certain experience. Please read the instructions carefully before using the kit. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
5) Do not use if a panel or packaging appears to be damaged.
6) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
7) Do not mix reagents of different batches. Please use the kit within the validity period.
8) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
9) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.

STORAGE

Store EU AFST at 2-8°C in the original packaging. Do not use the panels beyond the expiry date indicated on the label. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, EU AFST and material that has come into contact with the sample must be decontaminated and disposed of in accordance with guidelines used in the laboratory for decontamination and disposal of potentially infected material.

REFERENCES

1. ISO 16256:2021 "Clinical laboratory testing and in vitro diagnostic test systems — Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against yeast fungi involved in infectious diseases".
2. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs for antifungal agents, version 12.1, 2026. http://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals/.
3. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and agar dilution for yeasts, moulds and dermatophytes as recommended by EUCAST. Version 8.0, 2026. http://www.eucast.org.
4. The European Committee on Antimicrobial Susceptibility Testing. EUCAST antifungal MIC method for yeasts. EUCAST E.Def 7.4 October 2023.

Product	Packaging	Ref.
EU AFST	6 tests	783004

Table of Symbols

RUO	For Research Use Only
REF	Catalogue number
LOT	Batch code
	Do not reuse
	Fragile, handle with care
	Manufacturer
	Use by
	Contains sufficient for <n> tests
	Consult instructions for use
	Keep away from heat
	Temperature limits

Revision History

Revision	Release Date	Change Summary
0	09 Oct 2026	Initial release

Note: Minor typographical, grammar, and formatting changes are not included in revision history.

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EUCAST stands for European Committee on Antimicrobial Susceptibility Testing. These data have been made available at no cost by EUCAST and can be accessed freely on the EUCAST website: www.eucast.org. EUCAST recommendations are frequently updated and the latest versions are available at www.eucast.org.

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RUO

EU AFST

TEST RESULTS FORM

SAMPLE

DATE

Note: Please print **sufficient copies** of the form to satisfy the requirements of your test

		1	2	3	4	5	6	7	8	9	10	11	12
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E	Posaconazole	Positive control	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4
F	Anidulafungin	Positive control	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4
G	Micafungin	Positive control	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4
H	Flucytosine	Positive control	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16

[concentrations are in µg/mL]

For each panel:

- Read the results
- Indicate with "**+**" the microbial growth and with "**–**" the absence of microbial growth
- Determine the MIC and interpret according to the clinical breakpoint