SUSCEPTIBILITY TESTING OF YEASTS 2011

AGAR DIFFUSION METHOD WITH NEO-SENSITABS

- USING MUELLER-HINTON AGAR WITH 2% GLUCOSE AND 0.5 µG/ML METHYLENE BLUE.

INTRODUCTION

CLSI (formerly NCCLS) has established a standard method for Antifungal Disk Diffusion Susceptibility Testing of Candida species(1) including fluconazole and voriconazole.

In recent studies (2,3,4) the correlation of Neo-Sensitabs with CLSI (formerly NCCLS) reference disk diffusion and Broth Microdilution Method was demonstrated.

Neo-Sensitabs thus offers an economical method to perform reliable antifungal susceptibility testing.

The technique for susceptibility testing of yeast differs from traditional antibiotic susceptibility testing, and requires more experience, especially when reading the zone sizes. Experience results in greater accuracy.

Species identification is recommended in addition to susceptibility testing.

This folder is intended to give the user practical guidance on the procedure and how to read the zones.

ROSCO
DIAGNOSTICA

AGAR MEDIA:

MUELLER-HINTON WITH 2% GLUCOSE AND 0.5µG/ML METHYLENE BLUE

The addition of glucose provides a suitable fungal growth and the methylene blue dye enhances zone edge definition.

It is important that the medium supports adequate growth, otherwise zones obtained with Neo- Sensitabs will be larger than normal.

-INOCULUM – INOCULATION OF THE AGAR PLATE – INCUBATION TIME

I: Inoculum	Equivalent to 0.5 McFarland standard - the inoculum should result in semi-confluent growth with most Candida species isolates. IMPORTANT: Using a standardised inoculum is very important. Too heavy inoculum makes reading of the zones for the Imidazoles/Azoles difficult and may lead to susceptible strains being considered falsely resistant. Make sure that zone diameters of quality control strains are in range.
II: Inoculation of the agar plate	Dip a sterile cotton swab into the suspension – rotate several times. Remove excess fluid from the swab by pressing firmly against the inside wall above the fluid level. Inoculate the dried surface of the agar by streaking the cotton swap according to the standard method. Ensure an even distribution of the inoculum. The plate may be left open for 3 – 5 minutes, allowing excess moisture to be absorbed, before dispensing the Neo-Sensitabs onto the plate.
III: Incubation time	Incubation at 35 °C ± 1°C within 15 minutes after the Neo-Sensitabs have been applied. Reading of zones: 20 – 24 hours after incubation. Always examine the plates after overnight incubation – measure the inhibition zones if visible. If no visible growth with particular strains, reincubate for up to 24 hours more.

INTERPRETATION TABLE** (SYSTEMIC)

Ref. No.	Neo-Sensitabs	Potency	Code	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
82512	Fluconazole	25 µg	FLUCZ	≥ 19	18 – 15 (DD)	≤ 14	≤ 8	≥ 64
82312	Voriconazole	1 µg	VOR.1	≥ 17	16 - 14 (DD)	≤ 13	≤ 1	≥ 4
81012	Amphotericin B	10 µg	AMPH	≥ 15	14 – 10	< 10	≤ 1	≥ 2
81812	Itraconazole	10 µg	ITRAC	≥ 23	22 – 14 (DD)	< 13	≤ 0.12	≥ 1
81912	Ketoconazole	15 µg	KETOC	≥ 28	27 – 21	≤ 20	≤ 0.12	≥ 0.5
82412	Caspofungin*	5 µg	CASP5	≥ 16	15 – 13	≤ 12	≤ 0.25	≥ 1
82612N	Posaconazole	5 µg	POSAC	≥ 17	16 – 14(DD)	≤ 13	≤ 1	≥ 4

DD = dosis dependent

*** Potencies of antifungals, MIC breakpoints and zone breakpoints as recommended by CLSI for Fluconazole and Voriconazole (1). For Amphotericin B, Itraconazole, Ketoconazole and Posaconazole the MIC breakpoints recommended by CLSI are used.*

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INTERPRETATION TABLE. LOCAL TREATMENT

S	≥ 20 mm	≥ 15 mm	≥ 10 mm
I	12 – 19 mm	10 – 14 mm	-
R	≤ 11 mm	No zone	No zone
	Ciclopirox, Clotrimazole, Econazole, Miconazole, Terbinafine, Fluorocytosine 1µg (Shadomy agar)	Nystatin	Griseofulvin

READING OF THE ZONES

For the best reading the plates are held above a black, nonreflecting background which is illuminated with reflected light.

1. AZOLES (e.g. Fluconazole, Itraconazole, Voriconazole, Ketoconazole, Posaconazole)

For azoles the zones must be measured up to colonies of normal size (fig 1.)

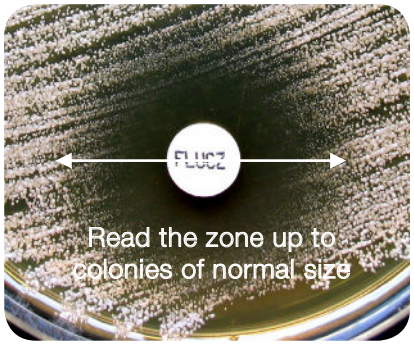

Fig. 1: Sensitive C. albicans

Fig. 2: Resistant C. albicans

There is often a zone of growth of partially inhibited colonies whose sizes are smaller nearer the tablet than at the edge of the real zone. These small and medium-size colonies are not resistant mutants.

2. POLYENES (e.g. Amphotericin B)

For Polyenes the clear zone with no visible growth is measured (fig 3.)

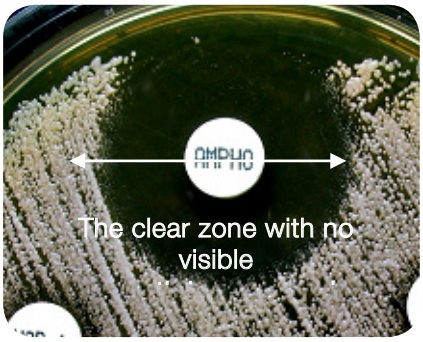

Fig 3. Sensitive C. albicans

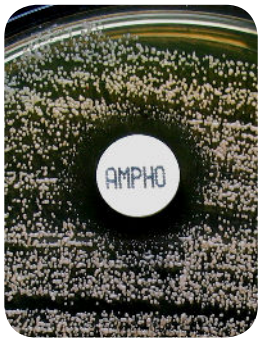

Fig. 4: Resistant C. albicans

Note: If there are colonies inside the zone, they must be considered resistant mutants.

QUALITY CONTROL

- INHIBITION ZONE IN MM. M H AGAR + 2 % GLUCOSE + METHYLENBLUE

	Fluconazole 25 µg	Voriconazole 1 µg	Itraconazole 10 µg	Ketoconazole 15 µg	Amphotericin B 10 µg	Posaconazole 5µg	Caspofungin 5µg
C. albicans ATCC 90028	28 – 39	31 – 42	21 – 30	31 – 42	20 – 27	24 – 34	15 – 22
C. parapsilosis ATCC 22019	22 – 33	28 – 37	19 – 26	26 – 35	22 – 29	25 – 36	13 – 23
C. krusei ATCC 6258	-	23 – 31	16 – 22	22 – 29	18 – 25	23 – 31	16 – 22
	Ciclopirox 50 µg	Clotrimazole 10 µg	Econazole 10 µg	Miconazole 10 µg	Terbinafine 30 µg	Nystatin 50 µg	
C. albicans ATCC 90028	22 – 30	28 – 36	19 – 26	22 – 29	9 – 9	20 – 26	
C. parapsilosis ATCC 22019	20 – 28	30 – 38	11 – 18	13 – 20	26 – 34	22 – 28	
C. krusei ATCC 6258	22 – 29	26 – 34	9 – 15	11 – 18	9 – 9	18 – 24	

THE WIDEST RANGE AVAILABLE

The currenct Neo-Sensitabs product range includes antifungal agents for systemic as well as for local treatment:

REF. No.	Product	Code	Potency µg
81012	Amphotericin B	AMPHO	10 *
81112	Ciclopirox	CICLO	50
81212	Clotrimazole	CTRIM	10
82412	Caspofungin	CASP5	5
81312	Econazole	ECONZ	10
82512	Fluconazole	FLUCZ	25
81512	Fluorocytosine 1 µg	FLU.1	1
81712	Griseofulvin	GRISE	25
81812	Itraconazole	ITRAC	10
81912	Ketoconazole	KETOC	15
82012	Miconazole	MICOZ	10
82212	Nystatin	NYSTA	50
87412	Terbinafine	TERBI	30
82312	Voriconazole	VOR.1	1
82612N	Posaconazole	POSAC	5

* = store at 2 - 8° C

Caspofungin
A new antifungal added to the Neo-Sensitabs range.

References:

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3. Espinel-Ingroff A. et al: Correlation between Neo-Sensitabs tablets on 3 media, NCCLS disk diffusion and broth microdilution methods for testing *Candida* spp. and *C. neoformans* with fluconazole and voriconazole. J. Clin. Microbiol. 45, 858-64, 2007.
4. Vanderbossche I. et al: Susceptibility testing of fluconazole by the NCCLS macrodilution method; E-test and disk diffusion methods. J. Clin. Microbiol, 40, 918-21, 2002.
5. NCCLS 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard M27-A2.
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11. Ochiuzzi ME et al : Correlation of E-test and Neo-sensitabs diffusion assays on Mueller-Hinton methylene blue agar with broth microdilution Reference method (CLSI-M27 -A2) for testing susceptibility to Cryptococcus neoformans to Amphotericin B and fluconazole. Med Mycology 48,893-896,2010.