RPMI AGAR

INTENDED USE

RPMI Agar: ready to use plates (ø 90 mm and 150 mm) for antifungal susceptibility test and MIC determination using Etest® fluconazole, ketoconazole, voriconazole, itraconazole, amphotericin B, flucytosine.

TYPICAL FORMULA (G/L)

RPMI 1640 with MOPS 0.165 M and L-glutamina	46.19
Glucose	20.00
Agar	15.00

pH 7,0 ± 0,2

MATERIALS REQUIRED BUT NOT SUPPLIED

Quality control organisms, sterile forceps, incubator, laboratory equipment.

DIRECTIONS

Use a template to position 5 strips on 150mm plate or 1-2 strips on 90mm plate.
Inoculum for yeast: Suspension in saline to 0.4 McFarland for Candida spp. and 1 McFarland for *C.neoformans*.
Incubation for yeast: 35°C/ ambient in bag/24-48 hours for Candida spp. e 48-72 hours for *C.neoformans*.

Inoculum for moulds: suspension of both conidia and hypae (mature growth 5-7days) in saline with Tween to 0.5 McFarland for Aspergillus spp. e 1 McFarland for *Fusarium* and *Rhizopus* spp.
Incubation for moulds: 35°C/moist/ ambient/24-72 hours, dependent on genus.

Guidelines:

Aspergillus spp.: 18-24 hours
Fusarium spp.: 35°C/24-48 hours, follow by room temperature 24-48 hours
Rhizopus spp.: 18-24 hours
For other species, extend the incubation time as needed, inspect plates daily for the inibition ellipse.

QUALITY CONTROL AND INTERPRETATION OF THE RESULTS: YEASTS

QUALITY CONTROL			*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019	*C. albicans* ATCC 90028 [1)]
(MIC μg/ml)	Amphotericin B	AP	0.5-2	0.25-1	0.125-0.5
	Flucytosine	FC	□32	0.064-0.5	0.25-2
	Fluconazole	FL	□256	1-8 [2)]	0.125-1
	Itraconazole	IT	0.25-1	0.064-0.5	0.064-0.25
	Ketoconazole	KE	0.25-1	0.032-0.125	0.008-0.032
	Voriconazole	VO	0.25-1	0.016-0.125	0.004-0.016

INTERPRETATION NCCLS, MIC (μg/ml) [3)]	**S**	**S-DD** [6)]	**I** [7)]	**R**
Fluconazole [4)]	≤8	16-32	-	≥64
Flucytosine	≤4	-	8-16	≥32
Itraconazole [5)]	≤0.125	0.25-0.5	-	≥1
Amphotericin B (*)	≤0.5 [8)]	-	-	-

Note

1. Use this strain to practise reading trailing end points of azoles i.e. growth of micro colonies in a discernable ellipse.
2. Occasionally macro colonies can grow up to 8 μg/ml.
3. NCCLS M27-A2, 2002.
4. Based on mucosal infections, *C. krusei* are assumed to be intrinsically resistant. The upper boundary for susceptibility is not known; S ≤4 μg/ml may also be useful.
5. Based on mucosal infections only.
6. Susceptible-Dose Dependent (S-DD) requires maximum blood level. For fluconazole, 400 mg/day may be required. For itraconazole, plasma levels of >0.5 μg/ml may be required for optimal response.
7. Uncertain intermediate category.
8. "if an amphotericin B MIC of >1 μg/ml is obtained,….then that isolate is likely resistant" (NCCLS M27-A2, p. 7, 2002).

QUALITY CONTROL AND INTERPRETATION OF THE RESULTS: MOULDS

QUALITY CONTROL and INTERPRETATION

Tentative QC (MCI μg/ml)

		C. parapsilosis ATCC 22019
Amphfotericin B	AP	0.25-2
Itraconazole	IT	0.064-0.25
Ketoconazole	KE	0.032-0.125

READING PRECAUTIONS

1. Practice by reading plates after varying periods of 1 to 3 days to become familiar with growth and end point appearance.
2. Make the first reading after 24 hours and the second at 48 hours. Slow growers may require up to 72 hours.
3. Read the MIC where the inhibition ellipse intersects the MIC scale.
4. Ignore filaments bending over into the ellipse, usually caused by overgrowth when incubation is prolonged.

WARNING

For in vitro diagnostic use only. RPMI Agar polates are for professional use only and they should be used by adequately trained personnel with knowledge of microbiological techniques in the laboratory. Observe approved biohazard precautions and aseptic techniques. Sterilize all biohazard waste before disposal.

STORAGE

RPMI Agar plates should be stored at 2-8°C protected from the light. When stored as directed the plates remain stable until the expiry date shown on the label. In case of remarkable changes of the colour or contamination discard the product and have a consulting advice from our technical assistance.

REFERENCES

- Szekely A. et al. Comparison of Etest and broth microdilution for antifungal drug susceptibility testing of moulds. JCM, vol. 37, no. 5, p. 1480-1483, 1999.
- Johnson E.M. et al. Lack of correlation of in vitro amphotericin B susceptibility testing with outcome in a murine model of aspergillus infection. JAC; vol. 45, no. 1, p. 85-93, 2000.
- Pfaller M.A. et al. In vitro susceptibility testing of filamentous fungi: comparison of Etest and reference microdilution methods for determining itraconazole MICs. JCM, vol. 38, no. 9, p. 3359-3361, 2000.
- NCCLS M38-P: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi; Proposed Standard (1998).
- Wanger et al. Comparison of Etest and NCCLS broth macrodilution method for antifungal susceptibility testing: Enhanced ability to detect amphotericin B-resistant *Candida* isolates. AAC, vol. 39, no. 11, p. 2520-2522, 1995.
- Pfaller et al. Evaluation of Etest method for determining fluconazole susceptibilities of 402 clinical yeast isolates by using three different agar media. JCM, vol. 36, no. 9, p. 2586-2589, 1998.

PACKAGING

54RPMI90	**RPMI AGAR 90 mm**	**box with 20 ready to use plates**
54RPMI15	**RPMI AGAR 150 mm**	**envelop with ready to use 5 plates**