

CM 21521 – RPMI AGAR W/MOPS 2% DEXTROSE (DOUBLE PACK)

INTENDED USE

For determining susceptibility of microorganisms to antifungal agents.

PRODUCT SUMMARY AND EXPLANATION

RPMI medium developed by Moore et al., at Roswell Park Memorial Institute is well known media used for cell culturing. The formulation is based on the RPMI series of media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins.

Invasive fungal infections have been increased over the past two decades. Due to the life threatening nature of these infections and reports of drug resistance, susceptibility testing of yeast pathogens has become very important. The CLSI have published a reference method for broth dilution antifungal susceptibility testing of Yeast. Also for use with the gradient-strip method when testing *Candida* spp. directly from colonies grown on nonselective media. RPMI Agar can be used to determine MIC values for various antifungal agents.

COMPOSITION

Ingredients	Gms / Ltr
Part I	
L-Asparagine	0.050
L-Aspartic acid	0.020
L-Cystine dihydrochloride	0.0652
L-Glutamic acid	0.020
L-Glutamine	0.300
Glycine	0.010
L-Histidine hydrochloride monohydrate	0.02096
L-Hydroxyproline	0.020
L-Isoleucine	0.050
L-Leucine	0.050
L-Lysine hydrochloride	0.040
L-Methionine	0.015
L-Phenylalanine	0.015
L-Proline	0.020
L-Serine	0.030
L-Threonine	0.020
L-Tryptophan	0.005



L-Tyrosine disodium salt	0.02883
L-Valine	0.020
D-Biotin	0.0002
D-Calcium Pantothenate	0.00025
Choline chloride	0.003
Folic acid	0.001
Inositol	0.035
Niacinamide	0.001
p-Amino benzoic acid (PABA)	0.001
Riboflavin	0.0002
Pyridoxine hydrochloride	0.001
Thiamine hydrochloride	0.001
Vitamin B12	0.000005
Calcium nitrate tetrahydrate	0.100
Potassium chloride	0.400
Magnesium sulphate anhydrous	0.04884
Sodium chloride	6.000
Sodium phosphate dibasic anhydrous	0.800
Glutathione reduced	0.001
Phenol red sodium salt	0.0053
MOPS Buffer, Free acid	34.500
L-Arginine hydrochloride	0.241
Part II	
D-Glucose	20.000
Agar	15.000

PRINCIPLE

The medium consists of Amino acids, vitamins and salts which provide essential nutrients. Glucose is the carbohydrate source. MOPS buffers the media. Agar acts as solidifying agent.

INSTRUCTION FOR USE

Part I: Dissolve 42.91grams of Part I in 500 ml distilled water.

Stir gently until the medium is completely dissolved. DO NOT HEAT.

Filter sterilize the medium using sterile membrane filter of 0.22 micron or less.



Part II: Dissolve 35 grams of Part II in 500 ml distilled water.

Mix well and heat to boiling to dissolve the medium completely.

Sterilize by autoclaving at 15 psi (121°C) for 15mins. Cool to 45-50°C.

Aseptically add filter sterilized Part I to Part II. Mix well before pouring into sterile Petri plates.

Note: The performance of this batch has been tested and standardized as per the current CLSI (formerly, NCCLS) document.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Part I: Cream to yellow homogeneous free flowing powder. Part II: Off-white to cream homogeneous free flowing powder.
Appearance of prepared medium	: Orangish red colour, clear to slightly opalescent gel forms in petri plate.
pH (at 25°C)	: 7.0 ± 0.1

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	MIC (Flucytosine) (µg/ml)	Incubation Temperature	Incubation Period
Candida parapsilosis	22019	10-100	Good-luxuriant	≥50%	0.06 - 0.5 µg	30-35°C	24-48 Hours
Candida krusei	6258	10-100	Good-luxuriant	≥50%	4 - 16 µg	30-35°C	24-48 Hours
Candida albicans	90028	10-100	Good-luxuriant	≥50%	0.5 - 2 µg	30-35°C	24-48 Hours
Candida albicans	24433	10-100	Good-luxuriant	≥50%	1 - 4 µg	30-35°C	24-48 Hours
Candida parapsilosis	90018	10-100	Luxuriant	≥70%	≤0.12 - 0.25 µg	30-35°C	24-48 Hours
Candida tropicalis	750	10-100	Luxuriant	≥70%	≤0.12 - 0.25 µg	30-35°C	24-48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is



opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. Vol.32No.17, December 2012 CLSI document M27-S4.

