RPMI 1640 Agar w/ MOPS & 2% Glucose w/o Sodium bicarbonate (Twin Pack) M1972

Intended use

RPMI 1640 Agar w/ MOPS & 2% Glucose w/o Sodium bicarbonate is used for determination of susceptibility of micro-organisms to antifungal agents.

Composition**

Ingredients	Gms / Litre
Part A	-
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 B	-
D-Glucose	20.000
Agar	15.000
Final pH (at 25°C)	7.0±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Part A : Suspend 42.91 grams of Part A in 500 ml distilled water. Stir gently until the medium is completely dissolved.DO NOT HEAT.Filter sterilise the medium using sterile membrane filter of 0.22 micron or less.
Part B : Suspend 35 grams of Part B in 500 ml distilled water. Mix well and heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure(121°C)for 15mins. Cool to 45-50°C.
Aseptically add filter sterilized Part A to Part B.Mix well before pouring into sterile Petri plates.
Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly,NCCLS)document.

Principle And Interpretation

RPMI-1640 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-1630 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(3). RPMI-1640 Agar can be used to determine MIC values for various antifungal agents. Amino acids, vitamins and salts provide essential nutrients. Glucose is the carbohydrate source. MOPS buffers the media. Agar acts as solidifying agent.

Type of specimen

Clinical samples : Pure cultures isolated from urine, stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. As antifungal susceptibility is carried with sensitivity disc, proper storage of the disc is desired which may affect the potency of the disc.
4. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

Performance and Evaluation

Performace of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Colour off Powder
Part A : Cream to yellow homogeneous free flowing powder.
Part B : Off-white to cream homogeneous free flowing powder.
Gelling
Firm, comparable with 1.5% agar gel.
Colour and Clarity of prepared medium
Yellowish green coloured clear to slight opalscent gel froms in Petri plates.
Reaction
Reaction of 4.29 grams of Part A in 50ml aqueous solution at 25°C. pH : 7.0±0.1

pH

6.90-7.10

Cultural response

Cultural characteristics observed after incubation at 30-35°C for 24 - 48 hours for fungal cultures.

Organism	Inoculum (CFU)	Growth	MIC(luc tosine) (µg ml)
Candida parapsilosis ATCC 22019	50-100	good-luxuriant	0.06 - 0.5 µg
**Candida krusei ATCC 6258*	50-100	good-luxuriant	4 - 16 µg
Candida albicans ATCC 90028	50-100	good-luxuriant	0.5 - 2 µg
Candida albicans ATCC 24433	50-100	good-luxuriant	1 - 4 µg
Candida parapsilosis ATCC 90018	50-100	luxuriant	<=0.12 - 0.25 µg
Candida tropicalis ATCC 750	50-100	luxuriant	<=0.12 - 0.25 µg

Note:(*) Quality control test of *Candida krusei* ATCC 6258 may or may not show MIC value, as highest concentration is 32 mcg/ml.

Storage and Shelf life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

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.

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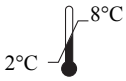
IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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