YEAST CARBON AGAR

INTENDED USE
Remel Yeast Carbon Agar is a solid medium recommended for use in qualitative procedures for differentiation of yeasts based on the ability to assimilate nitrogenous compounds.

SUMMARY AND EXPLANATION
Yeast Carbon Agar was developed by Wickerham to determine the ability of a yeast isolate to utilize an inorganic nitrogen source.[1,2] Wickerham determined certain yeasts require a vitamin-supplemented medium to demonstrate utilization of nitrogen compounds. The Delft plate method, recommended by Centers for Disease Control and Prevention (CDC), used Yeast Carbon Agar containing 2% Noble agar for differentiation of *Cryptococcus* spp.[3,4] A 1% peptone disk placed on one side of the agar plate served as a positive control, as all yeasts utilize peptone as a source of nitrogen.[5] A 1% potassium nitrate disk, placed on the other side of the agar plate, was used to detect nitrogen assimilation.

PRINCIPLE
Yeast Carbon Agar supplies amino acids, vitamins, trace elements, and salts which are necessary to support the growth of yeasts. Noble Agar is a pure, low ash agar which is the solidifying agent in this medium. The ability to assimilate nitrogen is determined by addition of various nitrogen sources to the yeast carbon base. Potassium nitrate is one of the most useful compounds for testing nitrogen assimilation. Peptone serves as a positive growth control.

REAGENTS (CLASSICAL FORMULA)*

Dextrose	10.0	g	Pyridoxine Hydrochloride	0.4	mg
Monopotassium Phosphate	1.0	g	Thiamine Hydrochloride	0.4	mg
Magnesium Sulfate	0.5	g	Zinc Sulfate	0.4	mg
Calcium Chloride	0.1	g	Ferric Chloride	0.2	mg
Sodium Chloride	0.1	g	ρ-Aminobenzoic Acid	0.2	mg
Inositol	2.0	mg	Riboflavin	0.2	mg
Methionine	2.0	mg	Sodium Molybdate	0.2	mg
Tryptophan	2.0	mg	Potassium Iodide	0.1	mg
L-Histidine Monohydrochloride	1.0	mg	Copper Sulfate	40.0	µg
Boric Acid	0.5	mg	Biotin	2.0	µg
Calcium Pantothenate	0.4	mg	Folic Acid	2.0	µg
Manganese Sulfate	0.4	mg	Noble Agar	20.0	g
Niacin	0.4	mg	Demineralized Water	1000.0	ml

pH 5.5 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
Auxanographic Method:[3]
1. Prepare the inoculum by making a suspension of the test isolate in sterile demineralized water. This suspension should not exceed the turbidity of a McFarland #1 turbidity standard or equivalent (REF R20411).
2. Add 1 ml of the yeast suspension to a tube of Yeast Carbon Agar which has been melted in a boiling water bath and cooled to 45-50°C.
3. Mix and dispense into a sterile petri dish and allow the medium to solidify.
4. Divide the plate in half and label one side potassium nitrate (KNO_3) and the other side peptone.
5. Place paper disks containing 1% KNO_3 and 1% peptone (positive control) on the agar surface, one on each half of the plate.
6. Incubate aerobically at 25-30°C with the agar surface up.
7. Examine for growth daily for 4 days.
8. In the absence of growth around the peptone disk, the test isolate results are invalid regardless of the presence or absence of growth around the 1% KNO_3 disk.

Alternate Method:
1. Melt two tubes of Yeast Carbon Agar. After cooling the media to 45-50°C, add 1% KNO_3 to one tube and 1% peptone (positive control) to the other tube.
2. Mix each tube and pour into separate sterile petri dishes.
3. Allow the medium to solidify and cool.
4. Inoculate each agar surface with a yeast suspension prepared in demineralized water as described above.
5. Incubate plates aerobically at 25-30°C.
6. Examine for growth daily for 4 days.
7. In the absence of growth on the medium containing peptone, the test isolate results are invalid regardless of the presence or absence of growth on the medium containing 1% KNO_3.

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Note: For each test isolate, inoculate a plate of Yeast Carbon Agar without KNO_3 or peptone as a control to test for carryover of nitrogen from the primary isolation medium. This plate should show no growth after incubation. If growth is present, the test is invalid due to carryover of nitrogen. In such instances, transfer a small amount of growth from this plate to another plate of the same medium (Yeast Carbon Agar) and repeat the test.

INTERPRETATION OF THE TEST

Auxanographic Method:
Positive Test - Growth around KNO_3 disk and peptone disk
Negative Test - Growth around peptone disk only

Alternate Method:
Positive Test - Growth on KNO_3 plate and peptone plate
Negative Test - Growth only on peptone plate

QUALITY CONTROL

All lot numbers of Yeast Carbon Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Candida albicans ATCC® 10231	Aerobic, 72 h @ 25-30°C	Growth
Cryptococcus neoformans ATCC® 34877	Aerobic, 72 h @ 25-30°C	Growth

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

IFU 9984, Revised January 11, 2010 Printed in U.S.A.

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