MIDDLEBROOK 7H9 BROTH w/ GLYCEROL

INTENDED USE
Remel Middlebrook 7H9 Broth w/ Glycerol is a liquid medium recommended for use in qualitative procedures for the isolation and cultivation of *Mycobacterium* species.

SUMMARY AND EXPLANATION
Middlebrook 7H9 Broth was first described by Middlebrook and Cohn in 1958 for the cultivation of mycobacteria.[1] The supplemental nutrients, glycerol, oleic acid, albumin, and dextrose were found to enhance the growth of most mycobacteria.[2,3] This medium is used as a basal medium to prepare inocula for biochemical identification tests and antibiotic susceptibility testing procedures.[4,5]

PRINCIPLE
Middlebrook 7H9 Broth w/ Glycerol contains inorganic compounds to supply potassium, sulfur, magnesium, and phosphorus which are necessary for the growth of mycobacteria. Inorganic copper, iron, zinc, and calcium are growth stimulators. Sodium citrate, when converted to citric acid, holds certain inorganic cations in solution. Ammonium sulfate is a nitrogen source and dextrose and glycerol are carbon sources. Albumin binds free fatty acids which may be toxic to *Mycobacterium* species. Catalase destroys toxic peroxides which may be present in the medium and catalyzes the reaction of iron with molecular oxygen. Biotin plays an important role in carboxylation and decarboxylation reactions, while pyridoxine is a precursor of enzymatic activity.

REAGENTS (CLASSICAL FORMULAE)*

Disodium Phosphate	2.5	g	Copper Sulfate	1.0	mg
Monopotassium Phosphate	1.0	g	Pyridoxine	1.0	mg
Ammonium Sulfate	0.5	g	Zinc Sulfate	1.0	mg
Monosodium Glutamate	0.5	g	Biotin	0.5	mg
Sodium Citrate	0.1	g	Calcium Chloride	0.5	mg
Magnesium Sulfate	0.05	g	Glycerol	2.0	ml
Ferric Ammonium Citrate	0.04	g	•ADC Enrichment	100.0	ml
			Demineralized Water	900.0	ml

pH 6.6 ± 0.2 @ 25°C

•ADC Enrichment:

Bovine Albumin (Fraction V)	50.0	g	Sodium Chloride	8.5	g
Dextrose	20.0	g	Catalase	30.0	mg
			Demineralized Water	1000.0	ml

*Adjusted as required to meet performance standards.

PROCEDURE
1. Follow established laboratory procedures for sample preparation, media inoculation, and incubation. Consult appropriate references for further instructions.[3-5]

QUALITY CONTROL
All lot numbers of Middlebrook 7H9 Broth w/ Glycerol 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
Mycobacterium fortuitum ATCC® 6841	CO_2, up to 14 days @ 33-37°C	Growth
Mycobacterium kansasii ATCC® 12478	CO_2, up to 14 days @ 33-37°C	Growth
Mycobacterium intracellulare ATCC® 13950	CO_2, up to 14 days @ 33-37°C	Growth
Mycobacterium scrofulaceum ATCC® 19981	CO_2, up to 14 days @ 33-37°C	Growth
Mycobacterium tuberculosis ATCC® 25177	CO_2, up to 14 days @ 33-37°C	Growth

BIBLIOGRAPHY

1. Middlebrook, G. and M.L. Cohn. 1958. Am. J. Public Health. 48:844.
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3. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology, A Guide for the Level III Laboratory. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
4. Howard, B.J., J.F. Keiser, A.S. Weissfeld, T.F. Smith, R.C. Tilton, and J. Comerford. 1994. Clinical and Pathogenic Microbiology. 2nd ed. Mosby, St. Louis, MO.
5. Clinical and Laboratory Standards Institute (CLSI). 2003. Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard, M24-A. CLSI, Wayne, PA.

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.

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