Middlebrook 7H9 Broth • Middlebrook 7H9 Broth with Glycerol • Middlebrook 7H9 Broth with Polysorbate 80 • Middlebrook ADC Enrichment

Intended Use

These media are used in qualitative procedures for the cultivation of mycobacteria.

Middlebrook 7H9 Broth dehydrated base (which may be supplemented with either glycerol or polysorbate 80) in combination with Middlebrook ADC Enrichment, and Middlebrook 7H9 Broth prepared tubes (containing ADC Enrichment) when supplemented with glycerol (2 mL/L) support the growth of mycobacteria, including *M. tuberculosis*. They are used primarily for growth of pure cultures of mycobacteria for use in laboratory studies.

These media and 7H9 Broth with Polysorbate 80 (0.5 mL/L) are used as subculture media for *Mycobacterium* species and in the preparation of inocula for drug susceptibility testing.

User Quality Control

Identity Specifications

Difco™ Middlebrook 7H9 Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.47% solution, soluble in purified water. Solution is very light amber, clear.
Prepared Appearance:	Colorless to very light amber, clear.
Reaction of 0.47% Solution at 25°C:	pH 6.6 ± 0.2

BBL™ Middlebrook ADC Enrichment

Appearance:	Very pale yellow, clear to trace hazy.
Reaction of Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Middlebrook 7H9 Broth with BBL™ Middlebrook ADC Enrichment

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium tuberculosis H37Ra	25177	10^2-3×10^2	Good
Mycobacterium kansasii, Group I	12478	10^2-3×10^2	Good
Mycobacterium scrofulaceum, Group II	19981	10^2-3×10^2	Good
Mycobacterium intracellulare, Group III	13950	10^2-3×10^2	Good
Mycobacterium fortuitum, Group IV	6841	10^2-3×10^2	Good

Summary and Explanation

Middlebrook and co-workers developed the 7H9 broth base formulation during the same time period in which they devised the 7H10 agar base.[1-3] Both media types support the growth of mycobacterial species when supplemented with nutrients such as glycerol, oleic acid, albumin and dextrose, except for *M. bovis* which is inhibited by glycerol. The albumin and dextrose, along with sodium chloride and catalase are provided by the Middlebrook ADC Enrichment.

Middlebrook 7H9 Broth may be additionally supplemented with polysorbate 80 for improved growth.[4,5] Middlebrook broths are commonly used in the preparation of inocula for antimicrobial assays, biochemical tests (arylsufatase and tellurite reduction) and for maintenance of stock strains.[4-6]

Principles of the Procedure

The large number of inorganic salts in this medium provide substances essential for the growth of mycobacteria. Sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. The albumin acts as a protective agent by binding free fatty acids, which may be toxic to *Mycobacterium* species. In the enriched medium, the albumin is heat-treated to remove lipase, which may release fatty acids from polysorbate 80;[7] catalase destroys toxic peroxides that may be present in the medium; dextrose is an energy source; and sodium chloride provides essential electrolytes. Supplementation with glycerol or polysorbate 80 enhances the growth of mycobacteria.

Formulae

Difco™ Middlebrook 7H9 Broth

Approximate Formula* Per 900 mL		
Ammonium Sulfate	0.5	g
L-Glutamic Acid	0.5	g
Sodium Citrate	0.1	g
Pyridoxine	1.0	mg
Biotin	0.5	mg
Disodium Phosphate	2.5	g
Monopotassium Phosphate	1.0	g
Ferric Ammonium Citrate	0.04	g
Magnesium Sulfate	0.05	g
Calcium Chloride	0.5	mg
Zinc Sulfate	1.0	mg
Copper Sulfate	1.0	mg

BBL™ Middlebrook ADC Enrichment

Approximate Formula* Per Liter		
Sodium Chloride	8.5	g
Bovine Albumin (Fraction V)	50.0	g
Dextrose	20.0	g
Catalase	0.03	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precaution[8]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

1. Suspend 4.7 g of the powder in 900 mL of purified water (containing 2 mL glycerol or 0.5 g polysorbate 80, if desired).
2. Autoclave at 121°C for 10 minutes.
3. Aseptically add 100 mL of Middlebrook ADC Enrichment to the medium when cooled to 45°C.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Middlebrook 7H9 Broth with appropriate supplements is primarily used for growth of pure cultures of mycobacteria for use in laboratory studies. Place inoculated tubes in a **BD GasPak**™ EZ container operated with a **GasPak** EZ carbon dioxide generator sachet, or other suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C for up to 8 weeks. Keep the caps of the tubes loosened for at least 1 week to permit circulation of CO_2, but tighten the caps thereafter to prevent dehydration, loosening briefly once a week to replenish CO_2.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary incubation. Cultures suspected to contain *M. avium* or *M. xenopi* exhbit optimum growth at 40-42°C.[9] Incubate a duplicate culture at 35-37°C.[9]

Expected Results

Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

Mycobacterial growth from the broth tubes can be utilized for additional laboratory test procedures as required.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Middlebrook. 1955. Fitzsimmons Army Hospital Report No. 1, Denver, Colo.
2. Middlebrook and Cohn. 1958. Am. J. Public Health. *48*:844.
3. Middlebrook, Cohn and Schaefer. 1954. Am. Rev. Tuberc. *70*:852.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Washington (ed.). 1985. Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
6. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Middlebrook, Cohn, Dye, Russel and Levy. 1960. Acta. Tuberc. Scand. *38*:66.
8. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
9. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.

Availability

Difco™ Middlebrook 7H9 Broth

AOAC SMWW

Cat. No. 271310 Dehydrated – 500 g

BBL™ Middlebrook 7H9 Broth

B512 CMPH2 MCM9

Cat. No. 295939 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H9 Broth with Glycerol

AOAC SMWW

Cat. No. 221832 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H9 Broth with Polysorbate 80

Cat. No. 297151 Prepared Tubes, 5 mL (C Tubes) – Pkg. of 10*

BBL™ Middlebrook ADC Enrichment

AOAC SMWW

Cat. No. 212352 Bottle, 100 mL – Pkg. of 6*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*