Middlebrook 7H10 Agar • Middlebrook and Cohn 7H10 Agar • Middlebrook OADC Enrichment

Intended Use

Middlebrook and Cohn 7H10 Agar, when supplemented with Middlebrook OADC Enrichment, is used in qualitative procedures for the isolation and cultivation of mycobacteria.

Summary and Explanation

Over the years, many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Lowenstein-Jensen Medium and

User Quality Control

Identity Specifications

Difco™ Middlebrook 7H10 Agar

Dehydrated Appearance:	Light beige to light beige with slight green tint, free-flowing, homogeneous.
Solution:	1.9% solution, soluble in purified water upon boiling. Solution is light to medium amber with slight green tint, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 1.9% Solution at 25°C:	pH 6.6 ± 0.2

BBL™ Middlebrook OADC Enrichment

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

Cultural Response

Difco™ Middlebrook 7H10 Agar with BBL™ Middlebrook OADC Enrichment

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 3-5% CO_2 for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Partial inhibition
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

Uninoculated Tube

Mycobacterium fortuitum ATCC™ 6841

Petragnani Medium. Dubos and Middlebrook were instrumental in the development of a number of formulations which contained oleic acid and albumin as key ingredients to aid in the growth of the tubercle bacilli and to protect the organisms against a variety of toxic agents.[1] Subsequently, Middlebrook and Cohn improved the formulation of oleic acid-albumin agar and obtained faster, more luxuriant growth of *Mycobacterium* species on their medium designated as 7H10.[2,3] The oleic acid and bovine albumin, along with sodium chloride, dextrose and catalase, are provided by the Middlebrook OADC Enrichment.

It has been reported that the 7H10 medium tends to grow fewer contaminants than the egg-based media commonly used for the cultivation of mycobacteria.[4]

Prepared plates of the complete medium are deep-filled to reduce the effects of drying during prolonged incubation.

Principles of the Procedure

Middlebrook and Cohn 7H10 Agar Base contains a variety of inorganic salts that provide substances essential for the growth of mycobacteria. The sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. Glycerol is an abundant source of carbon and energy.

Supplementation of the agar base is required in order to obtain mycobacterial growth. In the enriched medium, sodium chloride maintains osmotic equilibrium; oleic acid, as well as other long chain fatty acids, can be utilized by tubercle bacilli and plays an important role in the metabolism of mycobacteria; the primary effect of albumin is that of protection of the tubercle bacilli against toxic agents and, therefore, it enhances their recovery on primary isolation; dextrose is an energy source; and catalase destroys toxic peroxides that may be present in the medium. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

Formulae

Difco™ Middlebrook 7H10 Agar

Approximate Formula* Per 900 mL		
Ammonium Sulfate	0.5	g
Monopotassium Phosphate	1.5	g
Disodium Phosphate	1.5	g
Sodium Citrate	0.4	g
Magnesium Sulfate	25.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	1.0	mg
Copper Sulfate	1.0	mg
L-Glutamic Acid (sodium salt)	0.5	g
Ferric Ammonium Citrate	0.04	g
Pyridoxine Hydrochloride	1.0	mg
Biotin	0.5	mg
Malachite Green	250.0	µg
Agar	15.0	g

BBL™ Middlebrook OADC Enrichment

Approximate Formula* Per Liter		
Sodium Chloride	8.5	g
Dextrose	20.0	g
Bovine Albumin (Fraction V)	50.0	g
Catalase	0.03	g
Oleic Acid	0.6	mL

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

Precaution[9]

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 19 g of the powder in 900 mL of purified water containing 5 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes.
4. Aseptically add 100 mL of Middlebrook OADC Enrichment to the medium when cooled to 50-55°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[5] N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BD MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[5-8]

Following inoculation, keep plates shielded from light and place plates, medium side down, in a **BD GasPak™** EZ container operated with a **GasPak** EZ disposable carbon dioxide generator sachet, or other suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Tubes should be incubated in a slanted position at a 5° angle, permitting incubation of slant surfaces in a horizontal plane. Tubes and bottles should have screw caps loose for at least 1 week to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

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* exhibit optimum growth at 40-42°C.[5] Incubate a duplicate culture at 35-37°C.[5]

Expected Results

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

For reading plates or bottles, invert the containers on the stage of a dissecting microscope. Read at 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.

Record observations:[5]

1. Number of days required for colonies to become macroscopically visible.
2. Number of colonies (plates and bottles):
   No colonies = Negative
   Less than 50 colonies = Actual count
   50-100 colonies = 1+
   100-200 colonies = 2+
   Almost confluent (200-500) = 3+
   Confluent (more than 500) = 4+
3. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Acid-fast-stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.[5]

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. Dubos and Middlebrook. 1947. Am. Rev. Tuberc. *56*:334.
2. Middlebrook and Cohn. 1958. Am. J. Pub. Health. *48*:844.
3. Middlebrook, Cohn, Dye, Russell and Levy. 1960. Acta Tuberc. Scand. *38*:66.
4. Kubica and Dye. 1967. Laboratory methods for clinical and public health mycobacteriology. PHS Publication No. 1547. U.S. Government Printing Office, Washington, D.C.
5. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.
6. Cernoch, Enns, Saubolle and Richards. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
7. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, Sahm and Weissfeld. 2007. Bailey and Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
9. 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.

Availability

Difco™ Middlebrook 7H10 Agar

EP

Cat. No.	262710	Dehydrated – 500 g

BBL™ Middlebrook and Cohn 7H10 Agar

BS12 CMPH2 EP MCM9

United States and Canada

Cat. No.	221174	Prepared Plates (Deep Fill) – Pkg. of 20*
	295964	Prepared **I Plate**™ Dishes (Middlebrook 7H10 Agar and Middlebrook 7H10 Agar) – Pkg. of 20*
	220958	Prepared Slants, (A Tubes) – Pkg. of 10*
	220959	Prepared Slants, (A Tubes) – Ctn. of 100*
	297448	Prepared Slants, (C Tubes) – Pkg. of 10*
	297396	Prepared Slants, (C Tubes) – Ctn. of 100*
	297274	Prepared 1 oz. Transgrow-style Bottles – Ctn. of 100*

Europe

Cat. No.	254520	Prepared Plates – Pkg. of 20*

BBL™ Middlebrook OADC Enrichment

Cat. No.	212240	Bottle, 100 mL – Pkg. of 6*
	212351	Bottle – 500 mL*

Difco™ Glycerol

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

**Store at 2-8°C.*