Seven H11 Agars
Mycobacteria 7H11 Agar • Seven H11 Agar Base
Seven H11 Agar • Selective Seven H11 Agar
Seven H11 Agar with Aspartic Acid and Pyruvate

Intended Use

These media are used in qualitative procedures for isolation and cultivation of mycobacteria, especially *Mycobacterium tuberculosis*, from clinical and nonclinical specimens.

Summary and Explanation

Seven H11 Agar (also referred to as Middlebrook 7H11 Agar) was developed by Cohn et al. by the addition of casein hydrolysate to 7H10 Agar.[1] Seven H11 Agar provides enhanced

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Mycobacteria 7H11 Agar

Dehydrated Appearance:	Light beige to light beige with green tint, free-flowing, homogeneous.
Solution:	2.1 g/90 mL solution, soluble in purified water with 0.5% glycerol upon boiling. Solution is light yellowish green, very slightly to slightly opalescent.
Prepared Appearance:	Light yellowish green, very slightly to slightly opalescent.
Reaction of 2.1 g/90 mL with 0.5% Glycerol Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ Mycobacteria 7H11 Agar

Prepare the medium per label directions with added Middlebrook OADC enrichment. Inoculate and incubate at 35 ± 2°C with 3-5% and up to 10% 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	3×10^2-10^3	Good
Mycobacterium kansasii Group I	12478	3×10^2-10^3	Good
Mycobacterium scrofulaceum Group II	19981	3×10^2-10^3	Good
Mycobacterium intracellulare Group III	13950	3×10^2-10^3	Good
Mycobacterium fortuitum Group IV	6841	3×10^2-10^3	Good

Identity Specifications

BBL™ Seven H11 Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	19 g/900 mL solution, soluble in purified water with 0.5% glycerol. Solution is colorless to pale tan to light tan-green, slightly hazy to hazy.
Prepared Appearance:	Colorless to pale tan to light tan-green, slightly hazy to hazy.
Reaction of 19 g/900 mL with 0.5% Glycerol Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

BBL™ Seven H11 Agar Base

Prepare the medium per label directions with added Middlebrook OADC enrichment. Inoculate and incubate at 35 ± 2°C with 3-5% and up to 10% CO_2 for up to 21 days.

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

growth of fastidious, drug-resistant strains of *M. tuberculosis* that grow poorly (or not at all) on 7H10 Agar or other widely-used media.[1,2]

The Selective Seven H11 Agar is 7H11 Agar modified by the addition of four antimicrobial agents: polymyxin B, carbenicillin, amphotericin B and trimethoprim lactate. Mitchison et al. initially developed the medium to reduce the need for decontamination procedures.[3] They found that the alkaline agents used to reduce the growth of contaminating organisms inhibited some species of mycobacteria. McClatchy recommended reducing the concentration of carbenicillin used by Mitchison et al. to make the medium less inhibitory to mycobacteria.[4]

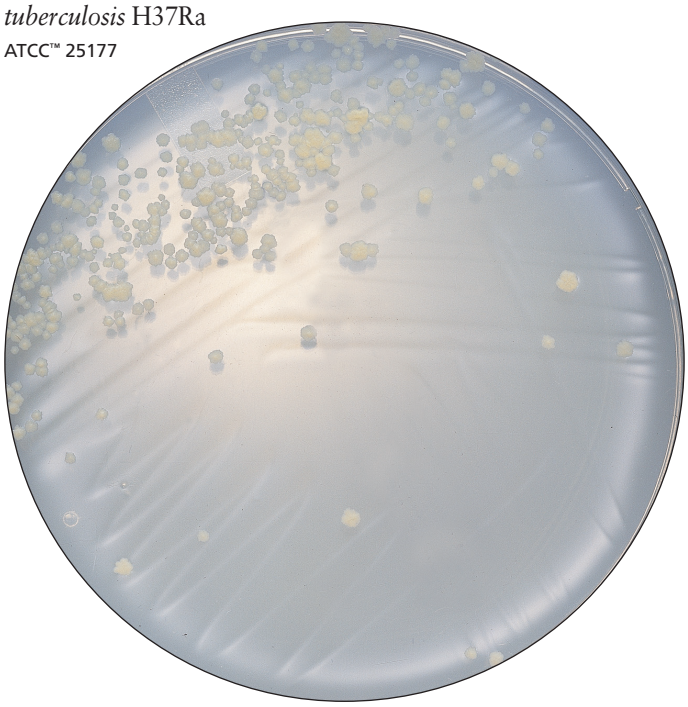
The addition of pyruvate to Seven H11 Agar has been recommended for specimens suspected of containing *Mycobacterium bovis*. The addition of aspartic acid has been recommended to enhance the production of niacin.[5]

Deep-filled plates are available to reduce the effects of drying during prolonged incubation.

Principles of the Procedure

Middlebrook 7H10 Agar is a defined medium consisting of oleic acid-albumin enrichment, glycerol, dextrose and inorganic compounds to supply the nutrients necessary to support the growth of mycobacterial species. Catalase destroys toxic peroxides that may be present in the medium. Malachite green acts as an inhibitory agent to provide partial inhibition of contaminating bacteria.

Mycobacterium tuberculosis H37Ra
ATCC™ 25177



Seven H11 Agar consists of 7H10 Agar supplemented with pancreatic digest of casein to enhance the growth of fastidious strains of *M. tuberculosis*.

The addition of antimicrobial agents to Seven H11 Agar improves the recovery of mycobacteria from specimens containing mixed flora.[2] Polymyxin B is a polypeptide antibiotic that selectively inhibits most species of gram-negative bacilli, including *Pseudomonas*, but not *Proteus* species.[6] Carbenicillin is a semi-synthetic penicillin effective against gram-positive and gram-negative bacteria, including strains of *Escherichia coli* resistant to other antimicrobial agents.[6] Amphotericin B is an antifungal antibiotic, and trimethoprim lactate is a synthetic antimicrobial agent that inhibits both gram-positive and gram-negative bacteria, including *Proteus* species.

With Seven H11 Agar containing aspartic acid and pyruvate, aspartic acid serves as a precursor for niacin synthesis by *M. tuberculosis* and *M. bovis*. All mycobacteria produce nicotinic acid (niacin). Because of a blocked metabolic pathway for the conversion of free niacin to nicotinic mononucleotide, *M. tuberculosis* accumulates niacin and excretes it into the culture medium, a function that differentiates it from most other mycobacterial species. Pyruvate enhances the recovery of *M. bovis*.

Formulae

Difco™ Mycobacteria 7H11 Agar

Approximate Formula* Per 900 mL	
Pancreatic Digest of Casein	1.0 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.4 g
Pyridoxine	1.0 mg
Biotin	0.5 mg
Ferric Ammonium Citrate	0.04 g
Ammonium Sulfate	0.5 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Magnesium Sulfate	0.05 g
Agar	15.0 g
Malachite Green	1.0 mg

BBL™ Seven H11 Agar Base

Approximate Formula* Per 900 mL	
Pancreatic Digest of Casein	1.0 g
Monosodium Glutamate	0.5 g
Sodium Citrate	0.4 g
Pyridoxine	1.0 mg
Biotin	0.5 mg
Ferric Ammonium Citrate	0.04 g
Ammonium Sulfate	0.5 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Magnesium Sulfate	0.05 g
Agar	13.5 g
Malachite Green	0.25 mg
Zinc Sulfate	1.0 mg
Copper Sulfate	1.0 mg
Calcium Chloride	0.5 mg

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

Precaution[7]

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 the powder in 900 mL of purified water containing 5 mL of glycerol:
   Difco™ Mycobacteria 7H11 Agar – 21 g;
   BBL™ Seven H11 Agar Base – 19 g.
2. Swirl to obtain a smooth suspension. For the **Difco** base, boil if necessary to completely dissolve the powder. For the **BBL** base do not boil.
3. Autoclave at 121°C for 15 minutes for the **Difco** base and 10 minutes for the **BBL** base.
4. Aseptically add 100 mL of Middlebrook OADC Enrichment to the medium when cooled to 50-55°C. Mix well.
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. 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 **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[8-11]

Following inoculation keep tubes shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes should have screw caps loose for the first 3 weeks 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 isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[4] Incubate a duplicate culture at 35-37°C.

For information on the niacin test, consult the ***BBL™ Quality Control and Product Information Manual for Plated and Tubed Media*** and other appropriate references.[2,8-11] **BBL™ Taxo™** TB Niacin Test Reagents (strips and control) may be used instead of the test reagents.

Expected Results

Cultures on Seven H11 Agar should be read within 5-7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations:[8]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Test all nonchromogenic mycobacteria on Seven H11 Agar with Aspartic Acid and Sodium Pyruvate for niacin production; only the rough nonchromogenic strains need to be tested for niacin. A culture must have at least 50-100 colonies with growth 3-4 weeks old. *M. tuberculosis* and the more rare *M. simiae* are usually niacin positive. Most other mycobacteria are niacin negative.

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. Cohn, Waggoner and McClatchy. 1968. Am. Rev. Respir. Dis. *98*:295.
2. Murray, Baron, Jogensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Mitchison, Allen, Carrol, Dickinson and Aber. 1972. J. Med. Mycol. *5*:165.
4. McClatchy, Waggoner, Kanes, Cernich and Bolton. 1976. Am. J. Clin. Pathol. *65*:412.
5. Kilburn, Stottmeier and Kubica. 1968. Am. J. Clin. Pathol. *50*:582.
6. Garrod and O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.
7. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Insitutes of Health. 2007. Biosafety in microbiology and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
8. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS, Centers for Disease Control, Atlanta, Ga.
9. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
10. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
11. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.

Availability

Difco™ Mycobacteria 7H11 Agar

Cat. No. 283810 Dehydrated – 500 g

BBL™ Seven H11 Agar Base

Cat. No. 212203 Dehydrated – 500 g

BBL™ Seven H11 Agar

BS12 CMPH2 MCM9

Cat. No. 221870 Prepared Plates (Deep Fill) – Pkg. of 10*
221391 Prepared Slants (A Tubes) – Pkg. of 10*
221392 Prepared Slants (A Tubes) – Ctn. of 100*
296105 Prepared Slants (C Tubes) – Pkg. of 10*
297704 Prepared Slants (C Tubes) – Ctn. of 100*

Japan
Cat. No. 252119 Prepared Plates (Deep Fill) – Pkg. of 20*

BBL™ Selective Seven H11 Agar

BS12 CMPH2 MCM9

Cat. No. 221868 Prepared Plates (Deep-fill) – Pkg. of 10*
297315 Prepared Slants (A Tubes) – Pkg. of 10*
297639 Prepared Slants (A Tubes) – Ctn. of 100*
297184 Prepared Slants (C Tubes) – Pkg. of 10*
297654 Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Seven H11 Agar with Aspartic Acid and Sodium Pyruvate

Cat. No. 221958 Prepared Slants (A Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H11 Agar//Selective 7H11 Agar

BS12 CMPH2 MCM9

Cat. No. 297250 Prepared Bi-Plate Dishes – Pkg. of 20*

Difco™ Glycerol

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

BBL™ Taxo™ TB Niacin Test Strips and Control

Cat. No. 231741 Vial – 25 strips*
231735 Cartridge, Control – 50 discs*

**Store at 2-8°C.*