

**QUALITY CONTROL PROCEDURES****I INTRODUCTION**

Selective 7H11 Agar is used for the isolation of mycobacteria. It contains antimicrobial agents to inhibit contaminating bacteria and fungi that may be present in specimens suspected of containing pathogenic mycobacteria.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Assure that the plates are free of moisture before inoculation.
 - b. For the *Mycobacterium* strains, inoculate plates with a dilution yielding 30–300 CFU to each plate and spread-inoculate using a sterile glass spreader. For all other organisms, use 5-h **Trypticase™** Soy Broth cultures and streak for isolation.
 - c. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
 - d. Include **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for the non-mycobacterial strains.
2. Examine plates after 7–21 days for growth and pigmentation.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Mycobacterium tuberculosis</i> , H37Ra	25177	Growth
* <i>Mycobacterium kansasii</i> , Group I	12478	Growth
* <i>Mycobacterium scrofulaceum</i> , Group II	19981	Growth
* <i>Mycobacterium intracellulare</i> , Group III	13950	Inhibition (partial to complete)
* <i>Mycobacterium fortuitum</i> , Group IV	6841	Growth
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)
<i>Candida albicans</i>	10231	Partial to complete inhibition
<i>Proteus mirabilis</i>	12453	Partial to complete inhibition
<i>Pseudomonas aeruginosa</i>	27853	Partial to complete inhibition

*Recommended organism strain for User Quality Control.

NOTE: Must be monitored by users, according to CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.6 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION**IV INTENDED USE**

Selective 7H11 Agar is used for the selective isolation and cultivation of pathogenic mycobacteria from specimens potentially contaminated with bacteria and fungi. The plates are deep-filled to reduce the effects of drying during prolonged incubation.

V SUMMARY AND EXPLANATION

During this century, many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Lowenstein-Jensen Medium and Petraghani 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}

Cohn et al. modified the 7H10 Agar formulation by the addition of one gram of pancreatic digest of casein per liter in order to enhance the growth of strains of *Mycobacterium tuberculosis* that were observed to grow poorly (or not at all) on 7H10 and other conventional isolation media.⁴ This formulation is designated as Seven H11 Agar.

In 1972, Mitchison et al. developed a selective medium by adding polymyxin B, amphotericin B, trimethoprim lactate and carbenicillin to Middlebrook and Cohn 7H10 Agar.⁵ Mitchison et al., in 1973, used the same combination of selective agents in Seven H11 Agar.⁶ In 1976, McClatchy et al. further modified the formula by reducing the carbenicillin concentration to 50 µg/mL.⁷

VI PRINCIPLES OF THE PROCEDURE

Selective 7H11 Agar contains a variety of inorganic salts which 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. The pancreatic digest of casein is a rich source of nitrogen for the growth of tubercle bacilli and provides a number of additional growth factors.¹ 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. Catalase destroys toxic peroxides that may be present in the medium. 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. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

The selective nature of Selective 7H11 Agar is due to the incorporation of carbenicillin, polymyxin B, amphotericin B and trimethoprim lactate in its formula. Carbenicillin is a synthetic penicillin which has a bactericidal effect on gram-negative bacteria, especially *Pseudomonas aeruginosa* and *Proteus* sp., by inhibiting cell wall synthesis.⁸ Polymyxin B is a polypeptide antibiotic that is inhibitory for gram-negative bacteria due to its damaging of their plasma membranes, which affects the permeability of the cells.⁸ Amphotericin B is a heptaene antibiotic which is active in the inhibition of fungi by altering the permeability of cell membranes, which contain cholesterol or ergosterol, thereby allowing permeation of various micromolecular compounds into the cell.⁹ Trimethoprim inhibits folic acid synthesis in gram-positive bacteria which require folic acid.¹⁰

VII REAGENTS

Selective 7H11 Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	1.0 g	Pyridoxine	1.0 mg
Magnesium Sulfate	0.05 g	Zinc Sulfate	1.0 mg
Ferric Ammonium Citrate	0.04 g	Copper Sulfate	1.0 mg
Sodium Citrate	0.4 g	Biotin	0.5 mg
Ammonium Sulfate	0.5 g	Calcium Chloride	0.5 mg
Monosodium Glutamate	0.5 g	Malachite Green	0.25 mg
Disodium Phosphate	1.5 g	Oleic Acid	0.06 mL
Monopotassium Phosphate	1.5 g	Glycerol	5.0 mL
Agar	13.5 g	Carbenicillin	50 mg
Sodium Chloride	0.85 g	Polymyxin B	200,000 units
Dextrose	2.0 g	Amphotericin B	10 mg
Bovine Albumin V	5.0 g	Trimethoprim Lactate	20 mg
Catalase	3.0 mg		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹¹⁻¹⁴ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

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.¹³

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times including up to 8 weeks for mycobacteriology media. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{15,16} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Selective 7H11 Agar (Deep Fill)

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surfaces should be smooth and moist, but without excessive moisture.

The test procedures are those recommended by the Centers for Disease Control (CDC) for primary isolation from specimens containing mycobacteria.¹⁷ N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. For detailed decontamination and culturing instructions, consult an appropriate reference.¹⁶⁻¹⁹

Following inoculation, keep plates shielded from light and place plates, medium side down, in a **BD GasPak™** EZ System, or other suitable carbon dioxide generating system.

Incubate plates at 35 ± 2°C in an aerobic, humidified atmosphere supplemented with carbon dioxide.

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.¹⁷ Incubate a duplicate culture at 35–37°C.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Plates may be read within 5–7 days after inoculation and once a week thereafter for up to 8 weeks.

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

Record observations:¹⁷

1. Number of days required for colonies to become macroscopically visible.
2. Number of colonies:
 - 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)

Stained smears may show acid-fast bacilli, which are reported only as “acid fast bacilli” unless definitive tests are performed.¹⁷

XI LIMITATIONS OF THE PROCEDURE

M. intracellulare may be inhibited on this medium, which contains carbenicillin.²⁰

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{15,16,21-24}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221868	BBL™ Selective 7H11 Agar (Deep Fill), Pkg. of 10 plates

XIII REFERENCES

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