



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Middlebrook and Cohn 7H10 Agar is a culture medium for the isolation and cultivation of 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. Inoculate plates with a dilution yielding 30-300 CFU to each plate and spread-inoculate using a sterile glass spreader.
c. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine plates after 7-21 days for growth.
3. Expected Results

Table with 3 columns: Organisms, ATCC™, Recovery. Rows include Mycobacterium tuberculosis, Mycobacterium kansasii, Mycobacterium scrofulaceum, Mycobacterium intracellulare, and Mycobacterium fortuitum.

*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

Middlebrook and Cohn 7H10 Agar is used in qualitative procedures for the isolation and cultivation of mycobacteria. 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 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.

VI PRINCIPLES OF THE PROCEDURE

Middlebrook and Cohn 7H10 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. 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.

VII REAGENTS

Middlebrook and Cohn 7H10 Agar

Approximate Formula* Per Liter Purified Water

Table listing reagents and their quantities: Magnesium Sulfate, Ferric Ammonium Citrate, Sodium Citrate, Ammonium Sulfate, Monosodium Glutamate, Disodium Phosphate, Monopotassium Phosphate, Agar, Sodium Chloride, Dextrose, Bovine Albumin V, Catalase, Pyridoxine, Zinc Sulfate, Copper Sulfate, Biotin, Calcium Chloride, Malachite Green, Oleic Acid, Glycerol.

*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"⁵⁻⁸ 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.⁷

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.^{9,10} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Middlebrook and Cohn 7H10 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 surface should be smooth and moist, but without excessive moisture.

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.¹¹ 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.¹⁰⁻¹³

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.¹¹ 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:¹¹

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.¹¹

XI LIMITATIONS OF THE PROCEDURE

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.^{9,10,14-17}

XII AVAILABILITY

Cat. No.	Description
221174	BBL™ Middlebrook and Cohn 7H10 Agar (Deep Fill), Pkg. of 20 plates

XIII REFERENCES

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