QUALITY CONTROL PROCEDURES

I INTRODUCTION

Seven H11 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. Streak the plates for isolation using a culture diluted to yield 10^3 – 10^4 CFUs.
   c. Incubate plates at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine plates after 7–21 days for growth and pigmentation.
3. Expected Results

Organisms	ATCC®	Recovery
**Mycobacterium tuberculosis* H37Ra	25177	Moderate to Heavy Growth
**Mycobacterium kansasii*, Group I	12478	Growth
**Mycobacterium scrofulaceum*, Group II	19981	Growth
**Mycobacterium intracellulare*, Group III	13950	Growth
**Mycobacterium fortuitum*, Group IV	6841	Growth

*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 at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Seven H11 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

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.[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 a study by Cohn, et al. of 96 clinical isolates, 13 failed to grow on the 7H10 medium in the first three weeks. Ten of the 13 cultures grew on Seven H11 in three weeks; the other three required an additional three-week incubation for the appearance of visible growth.[4]

VI PRINCIPLES OF THE PROCEDURE

Seven H11 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.

VII REAGENTS

Seven H11 Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	1.0 g	Bovine Albumin V	5.0 g
Magnesium Sulfate	0.05 g	Catalase	3.0 mg
Ferric Ammonium Citrate	0.04 g	Pyridoxine	1.0 mg
Sodium Citrate	0.4 g	Zinc Sulfate	1.0 mg
Ammonium Sulfate	0.5 g	Copper Sulfate	1.0 mg
Monosodium Glutamate	0.5 g	Biotin	0.5 mg
Disodium Phosphate	1.5 g	Calcium Chloride	0.5 mg
Monopotassium Phosphate	1.5 g	Malachite Green	0.25 mg
Agar	13.5 g	Oleic Acid	0.06 mL
Sodium Chloride	0.85 g	Glycerol	5.0 mL
Dextrose	2.0 g		

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

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

If excessive moisture is observed, invert 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 or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as **BD BBL™** specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.[9,10]

IX PROCEDURE

Material Provided: Seven H11 Agar (Deep Fill)

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

Test Procedure: The agar surface 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.[11] 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 BBL** MycoPrep™ Mycobacterial Specimen Digestion/ Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[9–12]

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

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 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

This medium is intended for primary isolation. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.[9–13]

XII PERFORMANCE CHARACTERISTICS

In a study conducted by Rastogi et al. at the Pasteur institute, drug susceptibility test results of 7H11 Agar were comparable with a radiometric method using the **BD BACTEC™** 460TB system. In addition, the investigation justified the choice of 7H11 Agar over Lowenstein-Jensen Agar for conventional drug susceptibility testing.[14]

XIII AVAILABILITY

Cat. No.	Description
221870	**BD BBL™** Seven H11 Agar (Deep Fill)

XIV REFERENCES

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Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA

EC REP Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland