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HARDY DIAGNOSTICS
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Instructions for Use

MIDDLEBROOK 7H11 AGAR, THIN POUR

Cat. no. SP57	Middlebrook 7H11 Agar, Thin Pour, 10x100mm SpaceSaver™ Plate, 18ml	15 plates/bag

INTENDED USE

Hardy Diagnostics Middlebrook 7H11 Agar, Thin Pour, is recommended for use in the isolation and cultivation of *Mycobacterium* species, using the microcolony method.

SUMMARY

In 1947 Dubos and Middlebrook formulated a media (7H9) containing albumin and oleic acid which enhanced the growth of tubercle bacilli, and protected the organisms against a variety of toxic agents.[5] Later, in 1958, Middlebrook and Cohn improved this first formulation and developed a media (7H10) which allowed more luxuriant, faster growth of *Mycobacterium* species.[9] Cohn, in 1968, incorporated casein hydrolysate into the 7H10 medium, and obtained a media that stimulated the growth of mycobacteria that would not otherwise grow on the 7H10 medium. This formulation was then designated 7H11 Agar, and is recommended over 7H10 Agar.[4,7]

Middlebrook 7H11 Agar contains inorganic compounds that supply essential growth stimulating inorganic salts as well as vitamins and necessary co-factors. Glycerol is provided as a source of carbon and energy for the tubercle organisms. Sodium citrate is converted to citric acid, which holds the inorganic cations in solution. Casein hydrolysate is incorporated into the 7H11 Agar as a growth stimulant for strains of drug resistant *Mycobacterium tuberculosis*.[2,8] Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin helps stimulate the revival of damaged organism as well as being involved in a variety of carboxylation and decarboxylation reactions. OADC Enrichment contains the following required additives: albumin to protect the tubercle bacilli against toxic agents; oleic acid, a fatty acid utilized in the metabolism of the organism; sodium chloride to maintain osmotic equilibrium; catalase to destroy any toxic peroxides in the medium; and dextrose as an energy source.

The microcolony method, first described by Welch, et al., in 1993, utilizes Middlebrook 7H11 Agar in a plate with a smaller fill amount than the standard plate.[12] In this procedure, plates are inoculated with a clinical specimen, sealed, incubated, and examined microscopically at regular intervals for the presence of *Mycobacterium* colonies. Welch, et al., also discovered that the conventional macroscopic method required an average of 23 days until detection of colonies was possible. However, the microcolony method on thinly poured 7H11 Agar plates detected the presence of colonies in an average of 11 days.[12] In addition to reducing the interval before colonies are detected, the microcolony method allows for the easy detection of mixed clinical specimens from mycological infections, and may yield a presumptive identification.[12]

FORMULA

Ingredients per 900ml of deionized water:*

Disodium Phosphate	1.5gm

Monopotassium Phosphate	1.5gm
Pancreatic Digest of Casein	1.0gm
L-Glutamic Acid	0.5gm
Ammonium Sulfate	0.5gm
Sodium Citrate	0.4gm
Magnesium Sulfate	50.0mg
Ferric Ammonium Citrate	40.0mg
Malachite Green	1.0mg
Pyridoxine	10.mg
Biotin	0.5mg
Glycerol	5.0ml
Agar	15.0gm

OADC Enrichment:	
Bovine Albumin	5.0gm
Beef Catalase	4.0gm
Dextrose	2.0gm
Sodium Chloride	0.85gm
Oleic Acid	50.0mg

Final pH 6.8 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be

considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.[1-3,6,7,11]

Method of Use:

1. According to test procedures recommended by the Centers for Disease Control (CDC), inoculate the Middlebrook 7H11 Agar with specimen, after decontamination and neutralization. Consult listed references for methods.[1-3,6,7,11]

2. After inoculation, fit the plate with a MycoSeal™ (Cat. no. SS9225). The MycoSeal™ allows for penetration of CO_2 to the plate, yet prevents excess moisture loss and dehydration of the plated media.

3. Incubate medium in a 5-8% CO_2 atmosphere at 35-37ºC., for up to four weeks. Protect from light.

4. Examine the sealed plates microscopically twice a week, for up to four weeks. To read plates, invert and place on the stage of a microscope, as focusing is performed through the bottom of the plate and agar. The correct plane of focus is easily determined by focusing on the streak lines still evident on the agar surface. Using transmitted light, scan the plate at 40X through the first two streaked quadrants. When detected, colonies are examined at 100X-400X to determine colony morphology. Microcolonies are characterized in descriptive terms relating to their margin and consistency.[12]

5. Consult appropriate references for recording the number of colonies, colony morphology, and for aid in the biochemical identification of acid-fast bacilli.[1,2,6,11]

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.[1-3,6,7,11]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Middlebrook 7H11 Agar requires incubation in a 5-10% CO_2 atmosphere in order to recover mycobacteria. For unknown reasons, mycobacteria are not recovered well from candle extinction jars.[7]

Keep inoculated media away from light or excessive heat, as exposure results in the release of formaldehyde in the media which may inhibit or kill mycobacteria.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, slides, decontamination supplies, MycoSeals™ (Cat. no. SS9225), applicator sticks, pipets, incinerators, CO_2 incubator, and microscopes, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Mycobacterium tuberculosis H37Ra ATCC® 25177	G	21 days	35°C	CO_2**	Growth; colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium kansasii Group I ATCC® 12478	G	21 days	35°C	CO_2**	Growth; colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth; colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth; colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium fortuitum Group IV ATCC® 6841	G	21 days	35°C	CO_2**	Growth; colonies visible in 4 days

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

** Atmosphere of incubation is enriched with 5-10% CO_2.

PHYSICAL APPEARANCE

Middlebrook 7H11 Agar should appear clear, slightly opalescent, and light amber with a green hue in color.

Mycobacterium kansasii Group I (ATCC® 12478) colonies growing on Middlebrook 7H11 Agar (Cat. no. SP57). Incubated in CO_2 for 12 days at 35ºC.

Mycobacterium scrofulaceum Group II (ATCC® 19981) colonies growing on Middlebrook 7H11 Agar (Cat. no. SP57). Incubated in CO_2 for 12 days at 35ºC.

Mycobacterium intracellulare Group III (ATCC® 13950) colonies growing on Middlebrook 7H11 Agar (Cat. no. SP57). Incubated in CO_2 for 12 days at 35ºC.

Mycobacterium fortuitum Group IV (ATCC® 6841) colonies growing on Middlebrook 7H11 Agar (Cat. no. SP57). Incubated in CO_2 for 12 days at 35ºC.

Uninoculated plate of Middlebrook 7H11 Agar (Cat. no. SP57).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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5. Dubos, R.J. and G. Middlebrook. 1947. *Am. Rev. Tuberc.*; 56:334-345.

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10. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

11. Vestal, A.L. 1975. *Procedures of the isolation and identification of mycobacteria*. DHEW (CDC 75-8230). Centers for Diseases Control. Atlanta, GA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]