IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

MIDDLEBROOK 7H9 BROTH

Cat. no. C32	Middlebrook 7H9 Broth, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. U32	Middlebrook 7H9 Broth, 500ml Polycarbonate Bottle, 500ml	1 each
Cat. no. C62	Middlebrook 7H9 Broth with Tween® 80, 16x125mm Tube, 5ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Middlebrook 7H9 Broth and Middlebrook 7H9 Broth with Tween® 80 are recommended for use in the cultivation of *Mycobacterium* spp. The media are also used for preparing dilutions of mycobacteria for antimicrobial testing.

Cat. no. U32 is not intended to be used for the diagnosis of human disease.

SUMMARY

In 1947, Dubos and Middlebrook formulated a tubercle bacilli growth enhancing medium to protect organisms against a variety of toxic agents.[5] This medium is named Middlebrook 7H9 Broth Base. The basal medium of 7H9 broth is supplemented with Middlebrook ADC Enrichment. The supplementation provides nutrients necessary for mycobacterial growth.

Bovine albumin, dextrose, and catalase are components of Middlebrook ADC Enrichment. Albumin acts as a protective agent and binds free fatty acids that may be toxic to *Mycobacterium* spp. Dextrose serves as an energy source. Toxic peroxides that may be present in the medium are destroyed by catalase. Additionally, the basal medium contains glycerol, biotin and sodium citrate. Glycerol provides an abundant source of carbon and energy for the tubercle organisms. Biotin helps stimulate the revival of damaged cells, and is involved in a variety of carboxylation and decarboxylation reactions. Sodium citrate, when converted to citric acid, holds inorganic cations in solution.

Middlebrook 7H9 Broth with Tween® 80 does not contain glycerol, but contains Tween® 80. Tween® 80 allows for the replication of microorganisms, and encourages the diffusion of new cells by wetting the surface of the tubercle bacilli. The compound is absorbed by the lipid portion of the bacterial surface and renders the tubercle bacilli dispersible in water.[13,14]

Middlebrook 7H9 Broth supplemented with glycerol is used for the maintenance of stock strains of pure cultures of mycobacteria for use in routine laboratory analyses. Middlebrook 7H9 Broth with Tween® 80 is used for the growth of *M. bovis* and in the preparation of inocula for the tellurite reduction test, useful in differentiating the *M. avium* complex (positive) from most other nonphotochromogenic mycobacteria (negative).

FORMULA

Ingredients per 900ml of deionized water:*

Disodium Phosphate	2.5gm
Monopotassium Phosphate	1.0gm
L-Glutamic Acid	0.5gm
Ammonium Sulfate	0.5gm
Sodium Citrate	0.1gm
Magnesium Sulfate	50.0mg
Ferric Ammonium Citrate	40.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
Glycerol	2.0ml

ADC Enrichment:

Bovine Albumin	5.0gm
Dextrose	2.0gm
Beef Catalase	3.0mg

In addition, Middlebrook 7H9 with Tween® 80 (Cat. no. C62) does not contain glycerol, but contains:

Dextrose	2.0gm
Tween® 80	0.5gm

Final pH 6.8 +/- 0.3 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, 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 dating 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 quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

For Cat. nos. C32 and C62.

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 blood precautions. 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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

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

For Cat. no. U32.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

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. Middlebrook 7H9 is primarily used for specimens usually from sterile body sites and for growth of pure cultures of mycobacteria for use in laboratory studies. Using aseptic techniques, inoculate a homogenized or centrifuged specimen directly to the medium. Consult listed references for methods.[1-3,6,7,11]

2. Incubate medium in a 5-10% CO_2 atmosphere at 35 +/- 2ºC., for up to eight weeks. Protect from light. Caps of tubes should be loosened for at least one week to allow circulation of CO_2. Tighten caps thereafter to prevent dehydration. Loosen caps briefly once a week in order to replenish CO_2.

3. Examine cultures within five to seven days after inoculation and weekly thereafter for up to eight weeks.

Mycobacterial growth from the broth can be used for additional laboratory test procedures, such as the tellurite

reduction test. It is recommended that biochemical testing be done for complete identification.

Tellurite Reduction Test:[15]

1. Inoculate tubes using a heavy inoculum from an actively growing solid culture.

2. Incubate tubes at 35ºC. for 7 days. Hand shake the tubes daily to encourage heavy growth. Tubes must be heavily turbid for testing. Note: If not heavily turbid by day 7, discard the tube and begin with a fresh culture.

3. Add two drops of a sterile 0.2% potassium tellurite solution to each tube and shake well to mix contents. Use only fresh potassium tellurite solution.

4. Reincubate the cultures at 35ºC. for 3 days. Do not shake the tubes during the second incubation period.

5. On day three, examine sedimented cells in each tube for formation of a black precipitate. Do not shake tubes during examination.

INTERPRETATION OF RESULTS

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

Turbidity in the bottom layer of the medium or throughout the tube indicates growth.

Tellurite ReductionTest Results:[15]

Positive - formation of a black precipitate of metallic tellurium in and around the sedimented cells.

Negative - growth of cells without a black precipitate. A light brown precipitate or gray precipitate is recorded as a negative result.

LIMITATIONS

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

Middlebrook 7H9 media require 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]

M. bovis is inhibited in the presence of glycerol.

Tellurite Reduction Test: If tubes do not show a heavy turbidity after 7 days incubation, repeat the test using a fresh, heavily turbid culture to minimize potentially questionable or false negative results.[15]

Most other rapidly growing mycobacteria also reduce tellurite to metallic tellurium within 3 days.[15]

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, applicator sticks, pipets, incinerators, CO_2 incubator, biological hoods, and microscopes, etc., as well as serological and biochemical reagents, such as 0.2% potassium tellurite solution, 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 Certificates of Analysis (CofA). The following organisms 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; turbidity at bottom or throughout tube
Mycobacterium kansasii Group I ATCC® 12478	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium fortuitum Group IV ATCC® 6841***	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube

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

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

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Middlebrook 7H9 Broth and Middlebrook 7H9 Broth with Tween® 80 should appear clear and colorless.

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.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

Mycobacterium kansasii Group I (ATCC® 12478) growing in Middlebrook 7H9 Broth (Cat. no. C32). Incubated in CO_2 for 21 days at 35ºC.

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4. Cohn, M.L., et al. 1968. *Am. Rev. Respir. Dis.*; 98:295.

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

6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

8. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

9. Middlebrook, G. and M.L. Cohn. 1958. *Am. J. Public Health*; 48:844-853.

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.

12. Welch, D.F., et al. 1993. Timely culture for mycobacteria which utilizes a microcolony method. *J. Clin. Microbiol.*; 31: 2178-2184.

13. Dubos, R.J. 1947. Experimental analysis of tuberculous infection. *Experientia*; 3, 45.

14. Dubos, R.J., et al. 1946. The effect of water soluble lipids on the growth and biological properties of tubercle bacilli. *Am. Rev. Tuberc.*; 54, 204.

15. Kilburn, J.O. V.A. Silcox, and G.P. Kubica. 1969. Differential identification of mycobacteria. V. The tellurite reduction test. *Am. Rev. Respir. Dis.*; 99(1):94-100.

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

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