IFU

Instructions for Use

MIDDLEBROOK 7H10 WITH HUMAN BLOOD

Cat. no. C42	Middlebrook 7H10 with Human Blood, 10ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Middlebrook 7H10 Agar with Human Blood is recommended for use in the isolation and cultivation of *Mycobacterium* spp., including *M. genavense*.

SUMMARY

Mycobacterium genavense is a newly described species of mycobacteria which is capable of causing disseminated disease in HIV infected individuals.[2,16] When isolated from clinical specimens, *Mycobacterium genavense* is most commonly found in blood.

Results of past studies indicated solid media were incapable of supporting growth of *M. genavense*. However, in 1992 Coyle reported growth of *M. genavense* on Middlebrook 7H11 supplemented with Mycobactin J.[15] Maier later reported the cultivation of the organism on Middlebrook 7H10 Agar supplemented with 10% human blood.[13] Studies conducted by Desmond found the human blood-supplemented medium to provide more luxuriant growth of *M. genavense*.[13,14]

Hardy Diagnostics Middlebrook 7H10 with Human Blood is supplemented with OADC Enrichment and 10% human blood. The medium contains a variety of inorganic salts, sodium citrate, vitamins, co-factors, oleic acid, albumin, biotin, catalase, and glycerol. Sodium citrate, when converted to citric acid, holds the inorganic cations in solution. Glycerol is provided as an abundant source of carbon and energy for the tubercle organisms. Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin and catalase help stimulate the revival of damaged organism. 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 10% human blood provides necessary components that promote the growth of *M. genavense*. The human blood components which promote growth of the organism have not yet been identified.[13]

FORMULA

Ingredients per 800ml of deionized water:*

Disodium Phosphate	1.5gm
Monopotassium Phosphate	1.5gm
L-Glutamic Acid (Sodium Salt)	0.5gm
Ammonium Sulfate	0.5gm
Sodium Citrate	0.4gm

Ferric Ammonium Citrate	40.0mg
Magnesium Sulfate	25.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
Malachite Green	0.25mg
OADC Enrichment	100.0ml
Human Blood	100.0ml
Glycerol	5.0ml
Agar	15.0gm

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

Final pH 6.6 +/- 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 (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat 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. Fixatives and preservatives should **not** be used. All specimens, except blood, should be refrigerated if transport to the laboratory is greater than one hour. Once recieved by the laboratory, specimens should be refrigerated until processing. Consult listed references for information on specimen collection.[1-3,6,7,11]

Method of Use:

1. Tissues or body fluids that have been aseptically collected usually do not require digestion and decontamination procedures.[17]

A. Normally sterile tissues may be ground in sterile 0.85% saline or 0.2% bovine serum albumin and then inoculated to the medium.[2]

B. Body fluids should be centrifuged at ≥3,000Xg. The sediment can then be inoculated to the medium.[2]

C. Specimens thought to be contaminated should first undergo digestion and decontamination procedures as recommended by the Centers for Disease Control (CDC). Consult listed references for methods.[1-3,6,7,11]

2. Once inoculated, tubes should be incubated in a 5-10% CO_2 atmosphere at 35-37ºC. for up to eight weeks.The caps should be kept loose for at least one week to allow circulation of carbon dioxide. Tighten caps, thereafter, to prevent dehydration. Loosen caps briefly once a week in order to replenish CO_2.

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

4. Observe for typical colonial growth and morphology. Consult listed references for identification procedures for specific organisms recovered. [2,3,6-9,11,13-15]

INTERPRETATION OF RESULTS

It is recommended that biochemical testing and/or chromatographic analysis be performed for definitive identification of microorganisms. Consult appropriate references for aid in the biochemical identification of acid-fast bacilli.[1,2,6,11]

LIMITATIONS

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 safety hoods, 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 fortuitum Group IV ATCC® 6841	G	21 days	35°C	CO_2**	Growth; colonies visible in 4 days

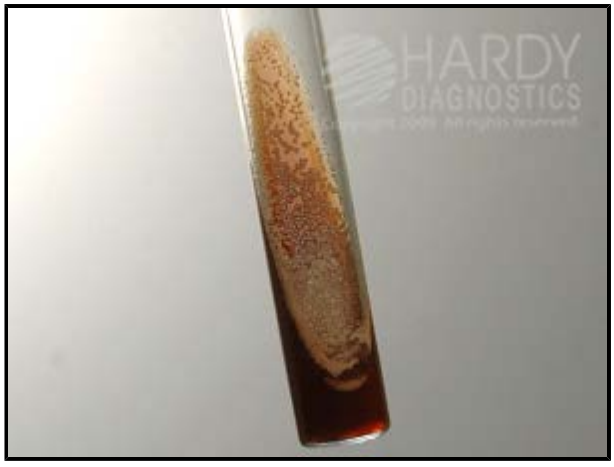
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

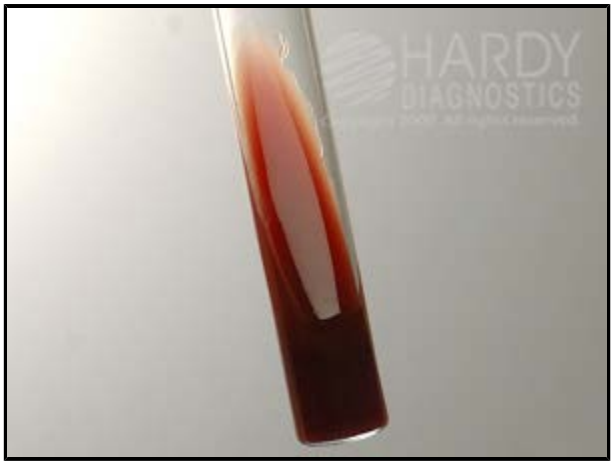
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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.

PHYSICAL APPEARANCE

Middlebrook 7H10 with Human Blood should appear opaque, and red in color.



Mycobacterium fortuitum Group IV (ATCC® 6841) colonies growing on Middlebrook 7H10 with Human Blood (Cat. no. C42). Incubated in CO_2 for four days at 35ºC.



Uninoculated tube of Middlebrook 7H10 with Human Blood (Cat. no. C42).

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IFU-10572[A]

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