IFU

Instructions for Use

CRITERION™ LOWENSTEIN MEDIUM BASE

Cat. no. C7380	CRITERION™ Lowenstein Medium Base	74.4gm
Cat. no. C7381	CRITERION™ Lowenstein Medium Base	500gm
Cat. no. C7382	CRITERION™ Lowenstein Medium Base	2kg
Cat. no. C7383	CRITERION™ Lowenstein Medium Base	10kg
Cat. no. C7384	CRITERION™ Lowenstein Medium Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Lowenstein Medium Base, when supplemented with egg and glycerol, is used for the cultivation, isolation, and differentiation of *Mycobacterium* spp.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

The original formulation of Lowenstein Jensen media was developed by Lowenstein who incorporated congo red and malachite green to inhibit unwanted bacteria.[6,7] The present formulation is based upon Jensen's modification. Jensen's version eliminates congo red and uses a moderate concentration of malachite green to prevent growth of the majority of contaminants surviving decontamination of the specimen. This formulation also encourages the earliest possible growth of mycobacteria.

Fresh eggs are added aseptically to this media. During heating, the egg albumin coagulates, thus providing a solid surface for inoculation. Nitrogen, fatty acids, and proteins are supplied by egg and asparagine. Glycerol serves as a carbon source and is favorable to the growth of the human type tubercle bacillus while being unfavorable to the bovine type. Malachite green acts as an inhibitory agent toward microorganisms other than mycobacteria.[8]

FORMULA

Gram weight per 600ml:	37.2gm/600ml
Potato Starch	30.0gm
Asparagine	3.6gm
Monopotassium Phosphate	2.4gm
Magnesium Citrate	0.6gm
Malachite Green	0.4gm

Magnesium Sulfate	0.24gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original medium to dark green-blue.

Store the prepared culture media at 2-8ºC.

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 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 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.

METHOD OF PREPARATION

1. Suspend 37.2gm of the dehydrated culture media in 600ml of distilled or deionized water containing 12ml of glycerol.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes. Cool to 45-60ºC. avoiding air bubbles.

4. Add the sterile base to 1 liter of a uniform suspension of fresh eggs prepared under aseptic conditions. Swirl gently to avoid introducing air into the suspension.

5. Dispense into sterile screw cap tubes or bottles. Arrange in a slanted position.

6. Place in an inspissator, waterbath or autoclave at 85ºC. for 45 minutes to coagulate medium.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. C21.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Lowenstein Jensen Media require incubation in a 5-10% CO_2 atmosphere in order to recover mycobacteria. Mycobacteria, for unknown reasons, are not recovered well from candle extinction jars.[4]

Protect the media from all sources of light, as malachite green is very photosensitive.

Selective media often inhibit, to some extent, specific strains of organisms for which they are designed to select.

The color of LJ Media may range from a pale-green to a dark blue-green. Do not use media that has turned yellow, as it will interfere with the interpretation of the pigmentation of mycobacteria. Most contaminating bacteria will turn the media blue.

Negative culture results do not rule out an active mycobacterial infection. Some factors responsible for unsuccessful cultures are:

1. The specimen was not representative of the infectious material (For example, saliva instead of sputum).

2. The mycobacteria were destroyed during digestion and decontamination of the specimen.

3. Gross contamination interfered with the growth of 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 autoclave, incinerators, and incubators, etc., 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 H3RV ATCC® 27294	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium kansasii Group I					Growth; colonies seen in 2

ATCC® 12478	G	21 days	35°C	CO_2**	weeks, mature in 3 weeks
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth; colonies seen 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

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Lowenstein Medium Base powder should appear homogeneous, free-flowing, and in medium to dark green-blue in color. The prepared media should appear opaque, and pale green in color.

REFERENCES

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

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

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

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

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

6. Lowenstein, E. 1933. *Ann. Inst. Pasteur.*; 50:161.

7. Lowenstein, E. 1931. *Zentralbl. Bakteriol. Parasetenkd. Infektionskr.*; 120:127.

8. Jensen, F. 1932. *Zentralbl. Bakteriol. Parasetenkd. Infektionskr.*; Abt. 1 Orig., 125:222.

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

HDQA 2207B [D]