IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

CRITERION™ EMB (EOSIN METHYLENE BLUE) AGAR, LEVINE

Cat. no. C5700	CRITERION™ EMB Agar, Levine	75gm
Cat. no. C5701	CRITERION™ EMB Agar, Levine	500gm
Cat. no. C5702	CRITERION™ EMB Agar, Levine	2kg
Cat. no. C5703	CRITERION™ EMB Agar, Levine	10kg
Cat. no. C5704	CRITERION™ EMB Agar, Levine	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ EMB Agar, Levine is recommended for use as a selective and differential medium for the isolation of gram-negative bacilli (including coliform organisms and enteric pathogens).

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

Eosin Methylene Blue (EMB) Agar was originally developed by Holt-Harris and Teague. Eosin dye was employed to inhibit the growth of gram-positive bacteria. Methylene blue was added as an indicator. Lactose and sucrose served as the nutrients. The production of acid, upon lactose- or sucrose-fermentation, resulted in the two dyes interacting to produce brown to blue-black colonies. This formulation gives sharp and distinct differentiation between colonies of lactose- and non-lactose-fermenting organisms. However, it does not discriminate between which carbohydrate (lactose or sucrose) is being fermented. *Yersinia enterocolitica*, which ferments sucrose but not lactose, will produce the same blue-black colony as lactose-fermenters.[1-6]

Levine modified the formula by omitting sucrose and doubling the level of lactose. The resultant medium gives excellent differentiation of *Escherichia coli* from *Enterobacter aerogenes*. This formulation, by eliminating sucrose, provides reactions that are more in parallel with MacConkey Agar.

EMB Agar, Levine has become the predominant enteric plating medium that utilizes dyes as selective agents. The American Public Health Association recommends its use in the microbiological examination of potable water, waste water, dairy products and foods.[8,9] The USP recommends its use in the performance of Microbial Limit Tests.[10]

FORMULA

Gram weight per liter:	37.5gm/L
Pancreatic Digest of Gelatin	10.0gm
Lactose	10.0gm
Dipotassium Phosphate	2.0gm
Eosin Y	0.4gm
Methylene Blue	65.0mg
Agar	15.0gm

Final pH 7.1 +/- 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 reddish-purple.

Store the prepared culture medium at 2-8ºC.

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

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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 37.5gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes. Avoid overheating.

4. Dispense as desired into sterile containers.

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. G25.

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.

Some gram-positive bacteria, such as enterococci, staphylococci and yeasts will grow on this medium and usually form pinpoint colonies.

Non-pathogenic, non-lactose-fermenting organisms will also grow on this medium.

Additional biochemical tests must be performed in order to distinguish these organisms from the pathogenic bacterial strains.

Serial inoculation may be required to assure adequate isolation of mixed flora samples.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC ® 25922	A	24hr	35°C	Aerobic	Growth; blue-black centered colonies with green metallic sheen
Salmonella enterica ATCC ® 14028	A	24hr	35°C	Aerobic	Growth; colorless to amber colonies
Enterococcus faecalis ATCC ® 29212	B	24hr	35°C	Aerobic	Pinpoint colonies at 24 hours

* 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 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

CRITERION™ EMB Agar, Levine powder should appear homogeneous, free-flowing, and reddish-purple in color. The prepared media should clear, and reddish-purple with slight orange tinge in color.

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.

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

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

5. MacConkey, A.T. 1905. Lactose-fermenting bacteria in faeces. *J. Hyg.*; 5:333-379.

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

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

8. *Standard Methods for the Examination of Dairy Products*, 9th ed. 1948.

9. Greenberg, A.E., et al., (ed.). 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. APHA, Washington, D.C.

10. *The United States Pharmacopeia*, 22nd rev. 1990. United States Pharmacopeial Convention, Rockville, MD.

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

HDQA 2207B [C]