IFU

Instructions for Use

EMB AGAR

Cat. no. G25	EMB Agar, Levine, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J22	Blood Agar / EMB Agar, Levine, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J52	CNA Agar / EMB Agar, Levine, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J58	MacConkey Agar / EMB Agar, Levine, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J71	Urine Quad Plate (EMB Agar, Levine / Xylose Lysine Deoxycholate (XLD) Agar, Modified / Simmons Citrate / Blood Agar, 5%), 15x100mm Biplate, 5ml/section	10 plates/bag
Cat. no. J79	All Purpose Quad Plate (EMB Agar / Mannitol Salt Agar (MSA) / Mycobiotic Agar / Blood Agar, 5%), 15x100mm Biplate, 5ml/section	10 plates/bag
Cat. no. P09	EMB Agar, Levine, Contact Plate, 15ml	10 plates/bag

INTENDED USE

Hardy Diagnostics EMB Agar formulations are recommended for use as selective and differential media for the isolation of gram-negative bacilli (including coliform organisms and enteric pathogens) from clinical and nonclinical specimens.

Cat. no. P09 is not intended to be used for the diagnosis of human disease.

SUMMARY

EMB (Eosin Methylene Blue) Agar (HHT) was developed by Holt-Harris and Teague as an alternative to Endo's medium for the isolation of enteric bacilli.[4] Eosin dye and methylene blue were employed to inhibit the growth of gram-positive bacteria; the dyes also serve as differential indicators for the products of fermentation. Lactose and sucrose provide a carbohydrate source. The production of acid from lactose- or sucrose-fermentation results in the eosin-methylene blue dye complex being taken up by bacterial cells to produce a brown to blue-black colony appearance. The HHT formulation provides a clear distinction between colonies of lactose and non-lactose-fermenting microorganisms. However, this formulation does not discriminate between carbohydrate utilization (lactose or sucrose). For example, *Yersinia enterocolitica* ferments sucrose and not lactose, but will produce the same blue-black colonies as lactose-fermenters.[2,3,5,7-9]

In 1918, Levine simplified the Holt-Harris and Teague formulation by omitting sucrose and doubling the quantity of lactose. The modification facilitated the differentiation of *Escherichia coli* from *Enterobacter aerogenes*, and paralleled the reactions of MacConkey Agar for better colony identification.

Historically, EMB Agar, Levine has become the predominant formulation for detecting fecal and non-fecal coliforms. The American Public Health Association recommends its use in the microbiological examination of potable water, waste water, dairy products and foods.[1,11] The USP recommends its use in the performance of Microbial Limit Tests, and the U.S. Food and Drug Administration (FDA) recommends the medium for enumerating *Escherichia coli* and

coliform bacteria.[9,12]

FORMULA

Ingredients per liter of deionized water:*

EMB Agar, Levine:	
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 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C 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

For Cat. nos. G25, J22, J52, J58, J71, J79.

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.

For Cat. no. P09.

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.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-3,5,8,9,11,12] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use: Allow plates to warm to room temperature. The agar surface should be dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface and streak for isolation with a sterile loop. Incubate plates aerobically at 35-37°C for 18-24 hours and protect from light. Examine plates for colonial morphology. If negative after 24 hours, reincubate an additional 24 hours.

Refer to the document " Contact Plate Media" for more information regarding the method of use for EMB Agar, Levine Contact Plate (Cat. no. P09).

INTERPRETATION OF RESULTS

Following incubation, examine plates for typical colonial morphology.

On EMB Agar, Levine, isolated colonies of lactose-fermenting bacteria appear brown to blue-black in color. *Escherichia coli* appears as large, blue-black colonies, often with a green metallic sheen. *Enterobacter* spp. present as brown to blue-black, mucoid colonies with no sheen. Non-lactose-fermenting colonies, such as *Shigella* spp. and *Salmonella* spp., appear transparent and colorless.

Consult listed references for further procedures for identification of isolates.[1,3,5,6,8,9]

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.

Some strains of *Salmonella* and *Shigella* may fail to grow on EMB Agar.[8]

Some gram-positive bacteria, such as enterococci, staphylococci, and yeast 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 pathogenic strains.

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

Some strains of *E. coli* may fail to produce a characteristic green metallic sheen; consequently, the green metallic sheen is not diagnostic for *E. coli*.[8]

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, swabs, applicator sticks, other culture media, incinerators, and incubators, 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	
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	Partial to complete inhibition; pinpoint colonies at 24 hours

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

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

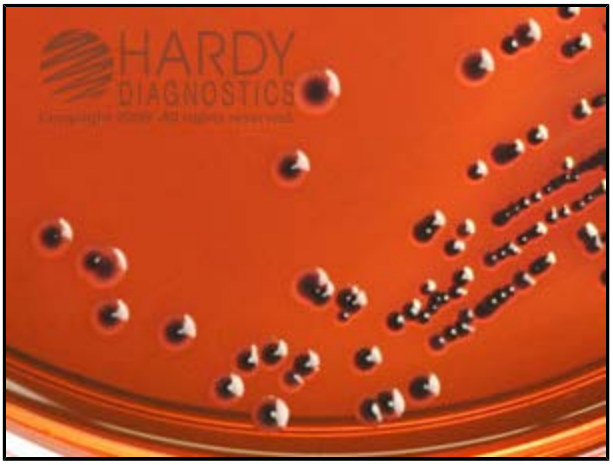
USER QUALITY CONTROL

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 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

EMB Agar, Levine should appear clear, and purple with a green-orange tinge in color. A slight precipitate is also apparent.

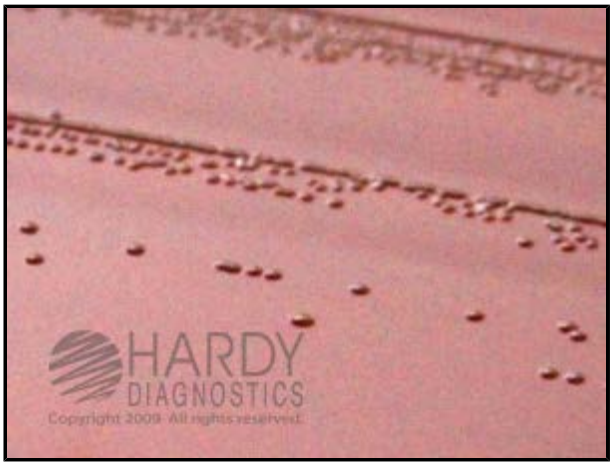
Escherichia coli (ATCC® 25922) colonies growing on EMB Agar, Levine (Cat. no. G25). Incubated aerobically for 24 hours at 35°C. Shot at an angle to show green sheen.



Escherichia coli (ATCC® 25922) colonies growing on EMB Agar, Levine (Cat. no. G25). Incubated aerobically for 24 hours at 35°C.

Salmonella enterica (ATCC® 14028) colonies growing on EMB Agar, Levine (Cat. no. G25). Incubated aerobically for 24 hours at 35°C.

Salmonella enterica (ATCC® 14028) colonies growing on EMB Agar, Levine (Cat. no. G25). Incubated aerobically for 24 hours at 35°C.



Enterococcus faecalis (ATCC® 29212) colonies growing on EMB Agar, Levine (Cat. no. G25). Incubated aerobically for 24 hours at 35°C.

Uninoculated plate of EMB Agar, Levine (Cat. no. G25).

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

2. 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.

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

4. Holt-Harris, J.E. and O. Teague. 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools.*J. Infect. Dis.*; 18:596.

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

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

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

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

9. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

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

11. The Official Compendia of Standards. *USP-NF*. United States Pharmacopeial Convention, Rockville, MD.

12. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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

HDQA 2207F [D]