IFU

Instructions for Use

CRITERION™ HEKTOEN ENTERIC (HE) AGAR

Cat. no. C5840	CRITERION™ Hektoen Enteric Agar	150gm
Cat. no. C5841	CRITERION™ Hektoen Enteric Agar	500gm
Cat. no. C5842	CRITERION™ Hektoen Enteric Agar	2kg
Cat. no. C5843	CRITERION™ Hektoen Enteric Agar	10kg
Cat. no. C5844	CRITERION™ Hektoen Enteric Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Hektoen Enteric (HE) Agar is a selective and differential medium used for the isolation and differentiation of gram-negative enteric bacilli.

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

King and Metzger developed HE Agar in an effort to increase the recovery of *Salmonella* and *Shigella* species over the previously formulated Salmonella-Shigella (SS) Agar.[6] This medium is particularly useful in the isolation of *Shigella* species.

The present formulation of HE Agar incorporates larger amounts of peptone in order to offset the inhibitory effect of bile salts. Also, sodium deoxycholate has been eliminated and the amount of bile salts reduced. Bile salts allow for the selective nature of HE Agar by inhibiting gram-positive organisms. Bile salts can also be toxic for some gram-negative strains. Salicin, sucrose, and lactose are the fermentable carbohydrates present. They provide optimal differentiation of enteric pathogens. Lactose and sucrose, in increased concentration, aid in the differentation of slow lactose-fermenters. Bromothymol blue and acid fuchsin (Andrade's) are added as acid-base indicators. The addition of ferric ammonium citrate and sodium thiosulfate enable the detection of H_2S, noted by the production of black centered colonies. Sodium thiosulfate serves as the sulfur source while ferric ammonium citrate serves as the indicator.

HE Agar is currently recommended as one of several plating media for the culture of Enterobacteriaceae from stool specimens. This is due to its moderately selective nature as well as for its differentiation properties.

FORMULA

Gram weight per liter:	76.0gm/L
Agar	14.0gm
Peptic Digest of Animal Tissue	12.0gm

Lactose	12.0gm
Sucrose	12.0gm
Bile Salts	9.0gm
Sodium Chloride	5.0gm
Sodium Thiosulfate	5.0gm
Yeast Extract	3.0gm
Salicin	2.0gm
Ferric Ammonium Citrate	1.5gm
Acid Fuchsin	0.1gm
Bromothymol Blue	0.064gm

Final pH 7.5 +/- 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 light tan-green.

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 FOR DEHYDRATED CULTURE MEDIA

1. Suspend 75.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely. Do not overheat.

3. **Do not autoclave**.

4. Cool to 45-50ºC. and aseptically add enrichments, if desired.

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. G63.

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.

Cultural growth may be delayed or inhibited by the presence of antimicrobial agents in the specimen. Additionally, antimicrobics may alter the characteristic appearance of the organism on the medium.

It is recommended that selective enrichment broths (GN or Selenite Cystine) be used in conjunction with selective plating media for optimal isolation of enteric pathogens.

The bile salts may crystallize over time. They appear as small spider-like puff-balls within the medium and do not affect the performance of the medium.

Colonies of *Proteus*, which may or may not be inhibited, may resemble *Salmonella* or *Shigella*.

The recovery of most *Shigella* and many *Salmonella* spp. from unpreserved stool specimens may be jeopardized if processing delays exceed 2-3 hours.

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

End users should anticipate the following typical performance characteristics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth; blue to blue-green colonies with black centers
Shigella flexneri ATCC® 12022	A	18-24hr	35°C	Aerobic	Growth; green to blue-green colonies
Enterococcus faecalis ATCC® 29212	B	18-24hr	35°C	Aerobic	Inhibition; may be slight growth of yellow colonies

Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial inhibition; may be slight growth of yellow to salmon colored colonies

* 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™ Hektoen Enteric (HE) Agar powder is homogeneous, free-flowing, and dark green in color. The prepared media should appear clear, and green in color.

REFERENCES

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

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

6. King, S. and Metzger, W.I. 1968. *Applied Microbiology*; 16:577-579.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

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