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HARDY DIAGNOSTICS
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Instructions for Use

HEKTOEN ENTERIC (HE) AGAR

Cat. no. G63	Hektoen Enteric (HE) Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J67	Columbia CNA Agar with Esculin / Hektoen Enteric (HE) Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J139	Hektoen Enteric (HE) Agar / SS Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

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

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.[7] 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 differentiation of enteric pathogens from 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 property.

FORMULA

Ingredients per liter of deionized water:*

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
Agar	13.5gm

Final pH 7.7 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. Products should not be used if there are any signs of contamination, deterioration, 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

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. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-6]

Method of Use: Medium should be brought to room temperature prior to inoculation. If material is being cultured directly from a swab, roll the swab over a small area of the surface edge. Streak the inoculum to obtain isolated colonies. A nonselective medium should also be inoculated. This increases the chance of recovery when the population of gram-negative organisms is low. It also provides indication of other organisms present in the specimen. Incubate plates for 18-24 hours at 35ºC. protected from light. If negative after 24 hours, reincubate for an additional 24 hours.

INTERPRETATION OF RESULTS

HE Agar is examined for typical colonial morphology after incubation. Fermentation of lactose, sucrose or salicin results in the production of acid which give rise to yellow-orange to salmon colored colonies. Colonies of *Salmonella* and *Shigella* spp. are green to bluish-green in color. *Salmonella* spp. that produce H_2S appear as blue-green colonies with black centers. H_2S producers form black-centered colonies in the presence of ferric ammonium citrate and sodium thiosulfate.

Consult listed references for further interpretation of growth and other identification tests to identify growth of organisms in this medium.[1-6]

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.

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.

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

The color of HE Agar may shift during shipment. This is normal and should not affect the performance of the medium. Placing plates in refrigerated conditions (2-8°C) upon receipt overnight may result in the medium returning to a normal appearance.

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 loops, other culture media, swabs, applicator sticks, 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	
Salmonella enterica ATCC® 14028	A	24hr	35°C	Aerobic	Growth; blue to blue-green colonies with black centers
Shigella flexneri ATCC® 12022	A	24hr	35°C	Aerobic	Growth; green to blue-green colonies
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibition; may be slight growth of yellow colonies
Escherichia coli ATCC® 25922**	B	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.

** Organism not tested on Cat. no. J67.

* 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

Hektoen Enteric (HE) Agar should appear brownish-green to green, clear to hazy, with dark precipitate evenly dispersed throughout the media.

Shigella flexneri (ATCC® 12022) colonies growing on Hektoen Enteric Agar (Cat. no. G63). Incubated aerobically for 24 hours at 35ºC.

Salmonella enterica (ATCC® 14028) colonies growing on Hektoen Enteric Agar (Cat no. G63). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in*

Enterococcus faecalis (ATCC® 29212) growth inhibited on Hektoen Enteric Agar (Cat no. G63). Incubated aerobically for 24 hours at 35ºC.

the Clinical Microbiology Laboratory, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Versalovic, J., et al. *Manual of Clinical Microbiology*. 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. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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

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

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

8. Centers for Medicare & Medicaid Services (CMS). Individualized Quality Control Plan (IQCP).

ATCC is a registered trademark of the American Type Culture Collection.

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

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