



BD™ Hektoen Enteric Agar (HE Agar)

INTENDED USE

BD Hektoen Enteric Agar is a moderately selective and differential medium for the isolation and cultivation of gram-negative enteric micro-organisms, especially for the isolation of *Shigella* and *Salmonella* species from fecal specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Hektoen Enteric Agar (HEA) was developed in 1967 by King and Metzger of the Hektoen Institute in order to improve the isolation of *Shigella* and *Salmonella* organisms when compared with other media frequently utilized at that time.^{1,2} This medium is considered to be moderately selective, and is particularly useful in the isolation of *Shigella* species. The present formulation differs from that of the original in that sodium desoxycholate has been eliminated and the concentration of bile salts reduced. Additionally, the peptone concentration has been increased in order to offset the inhibitory effects of the bile salts.³ This formulation is recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens.⁴⁻⁶

Bile salts render the medium selective, inhibiting Gram positive organisms and reducing growth of some gram-negative organisms other than *Salmonella* and *Shigella*. Lactose, sucrose and salicin are included for optimal differentiation by the color of the colonies and of the medium adjacent to the colonies. *Salmonella* and *Shigella* do not ferment these carbon compounds and thus do not cause a color change of the pH indicator system, while organisms fermenting one or several of these compounds to acids, e.g. *E. coli*, cause a color change to yellow or orange. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production by *Salmonella*. The pH indicator system consists of acid fuchsin and bromthymol blue.

REAGENTS

BD Hektoen Enteric Agar

Formula* Per Liter Purified Water

Peptic Digest of Animal Tissue	12.0 g	Sodium Chloride	5.0 g
Yeast Extract	3.0	Sodium Thiosulfate	5.0
Bile Salts	9.0	Ferric Ammonium Citrate	1.5
Lactose	12.0	Bromthymol Blue	0.064
Sucrose	12.0	Acid Fuchsin	0.1
Salicin	2.0	Agar	13.5

pH 7.6 +/- 0.2

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

PRECAUTIONS

IVD . For professional use only. ☒

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere. Examine plates after 18 to 24 h for amount of growth, colony size, pigmentation and selectivity. If negative, reincubate for an additional 18 to 24 hours.

Strains	Growth Results
<i>Salmonella</i> Typhimurium ATCC™ 14028	Growth good to excellent; colonies green to blue-green with black center
<i>Shigella flexneri</i> ATCC 12022	Growth good to excellent; colonies light green
<i>Shigella sonnei</i> ATCC 25931	Growth good to excellent; colonies light green
<i>Escherichia coli</i> ATCC 25922	Inhibition partial to complete; yellow-orange colonies, may have bile precipitates around the colonies, salmon to orange halos
<i>Enterococcus faecalis</i> ATCC 29212	Inhibition partial to complete; small yellow colonies, salmon to orange halos
Uninoculated	Green, nearly transparent

PROCEDURE

Materials Provided

BD Hektoen Enteric Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used for stool specimens from patients suspected to have a bacterial enteric infection and for similar materials (e.g. rectal swabs), and also for food samples (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak for isolation from this inoculated area.

A less selective medium such as **BD MacConkey II Agar** and selective liquid enrichment media, such as Selenite F Broth should also be inoculated to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. For a complete discussion on the isolation and identification of enteric pathogens from clinical specimens, refer to the procedures described in appropriate references.⁴

BD Hektoen Enteric Agar may be used as a medium for subculturing from Selenite F Broth. Incubate plates, protected from light, at 35 ± 2°C for 18 to 24 h. If negative, reincubate for an additional 18 to 24 hours.

Results

Typical colony morphology on **BD Hektoen Enteric Agar** is as follows:

Organisms	Growth Results
<i>E. coli</i>	Large, yellow to salmon color; some strains may be inhibited
<i>Enterobacter/Klebsiella</i>	Large, yellow to salmon color
<i>Proteus</i>	Variable, blue-green to blue or salmon, most strains with black center or completely black
<i>Salmonella</i>	Blue-green to blue; most strains with black center or completely

	black
<i>Shigella</i>	Green and moist, raised
<i>Pseudomonas</i>	Irregular, green to brown
Gram-positive bacteria	No growth to slight growth

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

This medium is one of the recommended standard media for the isolation of *Salmonella* and *Shigella* from human stool specimens or rectal swabs.³⁻⁶

A single medium is only rarely able to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella* and possibly for other enteric pathogens must be inoculated with the specimen.⁵

Proteus mirabilis colonies may resemble *Salmonella* on this medium.

Certain *Shigella* strains may need a 42 to 48 h incubation.

Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.

Colonies suspected of being *Salmonella* or *Shigella* must be confirmed and identified biochemically and serologically.⁴

REFERENCES

1. King, S., and W.I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens. I. Hektoen enteric agar. Appl. Microbiol. 16:577-578.
2. King, S., and W.I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen enteric agar with SS and EMB agar. Appl. Microbiol. 16:579-581.
3. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria. vol. I. Williams & Wilkins, Baltimore.
4. Bopp, C.A., Brenner, F.W., Fields, P.I., Wells, J.G., and N.A. Strockbine. 2003. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Kist, M. et al. 2000. Infektionen des Darmes. In: MIQ – Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik. Vol 9. Urban und Fischer, München, Jena.

PACKAGING/AVAILABILITY

BD Hektoen Enteric Agar

Cat. No. 254009 Ready-to-use plated media, 20 plates

Cat. No. 254075 Ready-to-use plated media, 120 plates

FURTHER INFORMATION

For further information please contact your local BD representative.



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