Hektoen Enteric Agar 84691.0500

Intended use

Medium for differential and selective isolation of *Salmonella* and *Shigella* species from enteric pathological specimens.

Formula ** - Composition in g/L

Ingredients	Gms / Litre
Proteose peptone	12.000
Yeast extract	3.000
Lactose	12.000
Sucrose	12.000
Salicin	2.000
Bile salts mixture	9.000
Sodium chloride	5.000
Sodium thiosulphate	5.000
Ferric ammonium citrate	1.500
Acid fuchsin	0.100
Bromothymol blue	0.065
Agar	15.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Instructions for preparation

Suspend 76.67 grams in 1000 ml purified / distilled water. Heat to boiling for 5-10 minutes to dissolve the medium completely. DO NOT AUTOCLAVE. Mix well and pour into sterile Petri plates.

Principle of the method and general information

Media that isolated a broader spectrum of enteric pathogens are less inhibitory to members of the non-pathogenic intestinal flora.Hektoen Enteric Agar was developed in 1967 by King and Metzger of the Hektoen Institute in order to increase the frequencies of isolation of *Shigella* and *Salmonella* organisms when compared with their recovery on other media frequently utilized in clinical laboratories at that time (1-3). Sodium deoxycholate has been replaced by bile salts in reduced concentration. This allows growth of *Shigella* as well as the Salmonellae. The peptone concentrations have been increased in order to offset the inhibitory effects of the bile salts (4). Hektoen Enteric Agar is currently recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens (5). Foods containing poultry, eggs or dairy products are the most frequent vehicles for foodborne Salmonellosis, and a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella* (6-9).

The increased concentration of carbohydrate and peptic digest of animal tissue helps to reduce the inhibitory effect of bile salts and indicators and allows good growth of *Salmonella* and *Shigella* species while inhibiting the normal intestinal flora. The medium contains three carbohydrates i.e lactose, sucrose and salicin for differentiation of enteric pathogens. The higher lactose concentration aids in the visualization of enteric pathogens and minimizes the problem of delayed lactose fermentation. Salicin is fermented by many coliforms including those that do not ferment lactose and sucrose. Combination of ferric ammonium citrate and sodium thiosulphate in the medium enables the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the formation of black centered colonies. The indicator system, consisting of acid fuchsin and bromothymol blue, has lower toxicity as compared to other enteric media, resulting in improved recovery of enteric pathogens. Hoben et al (10) further enhanced the selectivity of the medium by addition of novobiocin at a concentration of 15 mg/litre, which inhibits *Citrobacter* and *Proteus* species. Taylor and Schelhaut (11) found the medium valuable for differentiating pathogenic enteric organisms and for better growth of Shigellae.

Instruction for use

Inoculate the medium with fresh faeces suspended in Ringers Solution or inoculate directly with rectal swabs. Spread out the inoculum to obtain isolated colonies and incubate at 35-37°C for 18-24 hours. Further incubation will improve differentiation between *Salmonella* and *Shigella* . *Proteus* species may resemble *Salmonella* or *Shigella* ; hence further testing must be carried out for confirmation.

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Limitations

1. *Proteus* species may resemble *Salmonella* or *Shigella*, hence further testing is required to carry out confirmation.
2. Arizona may also be difficult to separate from *Salmonella* species.
3. Further biochemical testing is required to identify organisms isolated on this medium upto genera or species level

Quality Control

Appearance
Cream to yellow with tancast homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 7.67% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922	50-100	fair	20-30%	orange (may have bile precipitate)
Enterobacter aerogenes ATCC 13048	50-100	fair-good	30-40%	salmon-orange
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	>=50%	greenish blue may have black centres(H2S production)
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=50%	greenish blue may have black centres(H2S production)
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	>=50%	greenish blue may have black centres(H2S production)
Shigella flexneri ATCC 12022	50-100	luxuriant	>=50%	greenish blue
Escherichia coli ATCC 8739	50-100	Fair	20-30%	orange (may have bile precipitate)

Reference

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4.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I. Williams & Wilkins, Baltimore, Md.
5.Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R.H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
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9.Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
10.Giannella R. A., 1996, Salmonella. In: Barons Medical Microbiology (Baron S et al, eds.), 4th Ed., Univ. of Texas Medical Branch, Hoben D.A., Ashton D.H.A. and Peterson A.C., 1973, Appl. Microbiol., 21:126.
11.Taylor W.I. and Schelhaut, 1971, Appl. Microbiol., 21:32.

Storage conditions

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Ordering information

84691.0500	Hektoen Enteric Agar	Bottle of 0.5 Kg