Technical Information

Hektoen Enteric Agar

Product Code: DM 1467

Application: Hektoen Enteric Agar is a differential selective medium used for the isolation of *Shigella* and *Salmonella* species from enteric pathological specimens.

Composition**

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

Principle & Interpretation

Media that isolated a broad spectrum of enteric pathogens are less inhibitory to 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 rate of isolation of Shigella and Salmonella organisms when compared with their recovery on other media frequently used in clinical laboratories at that time [1-3]. Sodium deoxycholate has been replaced by bile salts with reduced concentration.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 culturing Enterobacteriaceae from stool specimens [5]. Foods comprising of poultry, eggs or dairy products are the most frequent sources for foodborne Salmonellosis, and different type 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 helps 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 helping in the differentiation process due to the formation of black centered colonies. Compared to other enteric media, the indicator system, consisting of acid fuchsin and bromothymol blue, has lower toxicity 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. 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 35-37^0 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 carries out for confirmation.

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

Methodology

Suspend 76.67 grams of powder media in 1000 ml distilled water. Shake well and heat to dissolve the medium completely. DO NOT AUTOCLAVE. Mix well and pour into sterile Petri plates.

Quality Control

Physical 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 Range 7.30-7.70

Cultural Response/Characteristics
DM1467: 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(H_2S production)
Salmonella Typhi ATCC 6539	50-100	Luxuriant	>=50%	Greenish blue may have black centres(H_2S production)
Salmonella Typhimurium ATCC 14028	50-100	Luxuriant	>=50%	Greenish blue may have black centres(H_2S 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)

Further Reading

1. King S. and Metzger W. I., 1967, Abstr. M99, p. 77. Bacteriol. Proc. Am. Soc. Microbiol.
2. King S. and Metzger W. I., 1968, Appl. Microbiol., 16:577. 3. King S. and Metzger W. I., 1968, Appl. Microbiol., 16:579.
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.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
8. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C
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.

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