Hektoen Enteric Agar Medium

MU467

Hektoen Enteric Agar Medium is recommended for differential and selective isolation of *Salmonella* and *Shigella* species from enteric pathological specimens in accordance to United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Protease peptone	12.000
Yeast extract	3.000
Lactose	12.000
Sucrose	2.000
Salicin	9.000
Bile Salts mixture (Equivalent to Bile Salt No. 3)	9.000
Sodium chloride	5.000
Sodium thiosulfate	5.000
Ferric ammonium citrate	1.500
Acid fuchsin	0.100
Bromothymol blue	0.065
Agar	14.000
Final pH	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 72.66 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Hektoen Enteric Agar, a selective and differential medium designed to isolate and differentiate members of the species *Salmonella* and *Shigella* from other *Enterobacteriaceae* and was developed by King and Metzger (1,2). When compared with other selective medium, this medium inhibits the growth of *Salmonella* and *Shigella* very slightly; thus giving high yields of these microorganisms, but at the same time inhibits accompanying gram positive and other microorganisms. This medium is recommended by United States Pharmacopoeia, 2009 for testing the presence of *Salmonella* in dietary supplements (3). This medium is recommended in testing of *Salmonella* in food sample by various standards (4,5,6)

Compared to other differentiating media commonly used in clinical laboratories, Hektoen Enteric Agar is efficient in increasing the isolation rate of *Salmonella* sp. Bile salts, bromthymol blue and acid fuchsin inhibit the growth of most Gram positive organisms. Lactose, salicin and sucrose, serves as fermentable source of carbohydrates to encourage the growth and differentiation of enteric bacteria. In this medium by increasing the carbohydrate and peptone content of the medium the inhibitory effect of bile salts and indicators are countered. Proteose peptone provides nitrogen, carbon, and amino acids required for organism growth. Yeast Extract is a vitamin source. Sodium chloride maintains the osmotic balance of the medium. Sodium thiosulfate provides a source of sulfur. Hektoen Enteric Agar can also detect the production of hydrogen sulfide gas, which turns parts of the medium black. Ferric ammonium citrate serves as iron source, which cause production of hydrogen sulfide from sodium thiosulphate and also aids in the visualization of hydrogen sulfide production by reacting with hydrogen sulfide gas to form a black precipitate.

Enterobacters that are capable of fermenting one or more of the carbohydrates produces yellow or salmon-orange coloured colonies like *Klebsiella pneumoniae* , that ferments lactose. Non-fermenters will produce blue-green colonies. Organisms that reduce sulfur to hydrogen sulfide will produce black colonies or blue-green colonies with a black center. *Salmonella* reduce sulfur to hydrogen sulfide, producing a black precipitate. *Micrococcus luteus* does not grow.

Quality Control

Appearance
Cream to yellow with tancast homogeneous free flowing powder

Gelling
Firm, comparable with 1.4% agar gel.

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

pH
7.30-7.70

Growth Promotion Test
Growth Promotion was observed in accordance with USP, after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response
MU467:

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature	Incubation period
Growth Promotion Test						
Salmonella Typhimurium ATCC 14028	50 -100	25 -100	>=50 %	blue-green with or without black centres	30 -35 °C	24 -48 hrs
Salmonella Abony NCTC 6017	50 -100	25 -100	>=50 %	blue-green with or without black centres	30 -35 °C	24 -48 hrs
Cultural Response						
Additional Microbiological testing						
Salmonella Enteritidis ATCC 13076	50 -100	25 -100	>=50 %	blue-green with or without black centres	30 -35 °C	24 -48 hrs
Salmonella Typhi ATCC 6539	50 -100	15 -40	30 -40 %	blue-green with or without black centres	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 25922	50 -100	0 -10	0 -10 %	orange (may have bile precipitate)	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 8739	50 -100	0 -10	0 -10 %	orange (may have bile precipitate)	30 -35 °C	24 -48 hrs
Shigella flexneri ATCC 12022	50 -100	25 -100	>=50 %	greenish blue	30 -35 °C	24 -48 hrs
Enterococcus faecalis ATCC 29212	>=10^3	0	0%		30 -35 °C	24 -48 hrs
Staphylococcus aureus ATCC 6538	>=10^3	0	0%		30 -35 °C	18 -48 hrs

Storage and Shelf Life

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

Reference

1. King, S., and W. I. Metzger. 1968. Appl. Microbiol. 16:577
2. King, S., and W. I. Metzger. 1968. Appl Microbiol. 16:579
3. United States Pharmacopoeia 2009, US Pharmacopoeial Convention, Inc.,Rockville, MD
4. Marshall, R. T. (ed.). 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. Downes F P and Ito K(Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C
6. AOAC, 2005, Bacteriological Analytical Manual, 18th ed., AOAC, Washington, DC

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