Hektoen Enteric Agar, w/ 1.4% agar

M467F

Hektoen Enteric Agar w/ 1.4% agar is used for the differential isolation of *Shigella* and *Salmonella* from food specimens in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Peptone	12.000
Yeast extract	3.000
Sodium Chloride	5.000
Bile salt mixture	9.000
Lactose	12.000
Sucrose	12.000
Salicin	2.000
Sodium thiosulphate	5.000
Ferric ammonium citrate	1.500
Bromothymol blue	0.065
Acid fuchsin	0.100
Agar	14.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 75.67 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Mix well and pour into sterile Petri plates.

Principle And Interpretation

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). Hektoen Enteric Agar w/ 1.4% agar is used for the differential isolation of *Salmonella* and *Shigella* from food specimens in accordance with FDA BAM, 1998.

The increased concentration of carbohydrates and peptic digest of animal tissue helps to reduce the inhibitory effect of bile salts and indicators thus allowing the 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 indicated by the black coloured 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. Low concentration of bile salts allows the growth of *Shigella* and Salmonellae. Higher concentration of peptone minimizes the inhibitory effects of the bile salts (4, 5).

Grow suspected sample in TetraThionate broth overnight. Mix (vortex, if tube) and streak 3 mm loopful (10 µl) incubated TT broth on Hektoen Enteric Agar w/ 1.4% agar. Appearance of blue-green to blue colonies with or without black centers indicates the presence of *Salmonella* . Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

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

Reaction
Reaction of 7.57% 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°C for 22-26 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
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)

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

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