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Hektoen Enteric Agar (DM422)

Intended Use

Hektoen Enteric Agar (DM422) is recommended for differential and selective isolation of gram-negative enteric microorganisms, especially *Salmonella* and *Shigella* species, from a variety of clinical and non-clinical specimen.

Product Summary and Explanation

For the isolation of enteric pathogens, through the years many media have been devised differing in their degree of selectivity for the pathogenic species. Some were formulated to isolate and differentiate *Shigella* species whereas others were formulated for the selective isolation of the *Salmonellae*. Media that isolated a broader spectrum of enteric pathogens were less inhibitory to members of the non-pathogenic intestinal flora. In 1967, Hektoen Enteric Agar was developed by King and Metzger of the Hektoen Institute so as 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] This medium is considered to be moderately selective, and is particularly useful in the isolation of *Shigella* species. Present formulation differs from that of the original in that sodium desoxycholate has been replaced by reduced concentration of bile salts. In addition, the concentrations of peptone 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]

Principles of the Procedure

Hektoen Enteric Agar contains increased concentration of carbohydrate and peptic digest of animal tissue which 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 also, contains three carbohydrates i.e lactose, sucrose and salicin for optimal 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. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the production 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*.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	12.00
Yeast extract	3.00
Lactose	12.00
Sucrose	12.00
Salicin	2.00
Bile salts mixture	9.00
Sodium chloride	5.00
Sodium thiosulphate	5.00
Ferric ammonium citrate	1.50
Acid fuchsin	0.10

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Bromothymol blue	0.065
Agar	15.00
Final pH: 7.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 76.67 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. DO NOT AUTOCLAVE.
4. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow with tan cast homogeneous free flowing powder
Prepared Medium	Green coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 7.67% Solution	pH : 7.5 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

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

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	*Escherichia coli ATCC 25922*	50 - 100	fair	20-30%	orange (may have bile precipitate)
2.	*Enterobacter aerogenes ATCC 13048*	50 - 100	fair-good	30-40%	salmon-orange
3.	*Enterococcus faecalis ATCC 29212*	>=10^3	inhibited	0%	--
4.	*Salmonella Enteritidis ATCC 13076*	50 - 100	good-luxuriant	>=50%	greenish blue may have black centres (H_2S production)
5.	*Salmonella Typhi ATCC 6539*	50 - 100	good-luxuriant	>=50%	greenish blue may have black centres (H_2S production)
6.	*Salmonella Typhimurium ATCC 14028*	50 - 100	good-luxuriant	>=50%	greenish blue may have black centres (H_2S production)
7.	*Shigella flexneri ATCC 12022*	50 - 100	good-luxuriant	>=50%	greenish blue
8.	*Escherichia coli ATCC 8739*	50 - 100	fair	20-30%	orange (may have bile precipitate)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the medium with fresh faeces suspended in Ringers Solution or inoculate directly with rectal swabs.

2. Spread out the inoculum to obtain isolated colonies and incubate at 35-37°C for 18-24 hours.
3. Further incubation will improve differentiation between *Salmonella* and *Shigella*.
4. *Proteus* species may resemble *Salmonella* or *Shigella*; hence further testing must be carried out for confirmation.

Results

1. 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.
2. 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.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. *Proteus* species may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Hektoen Enteric Agar
Product Code : DM422
Available Pack sizes : 100gm/ 500gm

References

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11. Taylor W.I. and Schelhaut, 1971, Appl. Microbiol., 21:32.

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Further Information

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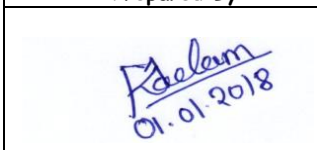	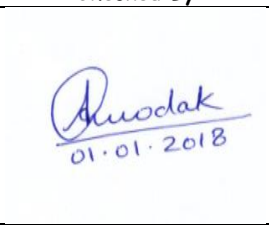	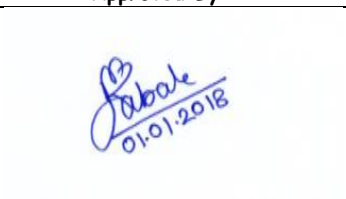
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