Hektoen Enteric Agar

M467

Intended use

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

Directions

Suspend 76.67 grams in 1000 ml 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

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 Sodium deoxycholate has been replaced by bile salts in reduced concentration.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 proteose peptone 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.

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.

Type of specimen

Clinical samples : Blood, urine, faeces ; Foods , water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines(12,13).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines(6,7,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.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.
2. Since the medium is selective it must be used in conjunction with other media.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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
M467: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Escherichia coli ATCC 25922 (00013*)	50-100	fair	20-30%	orange (may have bile precipitate)
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	fair-good	30-40%	salmon-orange
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0%	
Salmonella Enteritidis ATCC 13076 (00030*)	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 (00031*)	50-100	luxuriant	>=50%	greenish blue may have black centres(H_2S production)
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	>=50%	greenish blue
Escherichia coli ATCC 8739 (00012*)	50-100	Fair	20-30%	orange (may have bile precipitate)

Key : *Corresponding WDCM numbers.
- Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (12,13).

Reference

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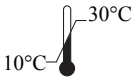
Revision : 03/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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