AOAC
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HEKTOEN Enteric Agar

Selective agar proposed by KING and METZGER (1968) for detecting and isolating pathogenic intestinal bacteria including Salmonella and Shigella in various materials such as faeces, foodstuffs, etc.

IVD

In Vitro Diagnostic Medical Device –

For professional use only

CE

Version 06-01-2011
Merck KGaA, 64271 Darmstadt

See also General Instruction for Use „How to use Dehydrated Culture Media"

For MSDS, warnings and precautions see our website: www.merck-chemicals.com

Principle

Microbiological method.

General Information

When compared with other selective culture media (e.g. SS Agar, BPL Agar and Bismuth Sulfite Agar), HEKTOEN* enteric agar has the advantage that it only slightly inhibits the growth of Salmonella and Shigella thus giving high yields of these microorganisms, but at the same time ensures adequate inhibition of accompanying microorganisms (KING and METZGER 1968, TAYLOR and SCHELHART 1971, BISCIELLO and SCHRADE 1974).

Principle

Microbiological method

Mode of Action

Lactose-positive colonies have a clearly different colour from lactose-negative colonies due to the presence of the two indicators bromothymol blue and acidic fuchsin. This colour difference is also observed for colonies, which can only slowly ferment lactose due to the presence of sucrose and salicin. These reactive compounds can be fermented more easily - false-positive pathogenic results are thus avoided. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H_2S-positive colonies to become black in colour. The mixture of bile salts suppresses the growth of most of the accompanying microorganisms.

HOBEN et al. (1973) recommended addition of 10-20 µg novobiocin/ml to the medium to improve its selectivity i.e. to inhibit Citrobacter and Proteus colonies which resemble those of Salmonella (black centre).

Typical Composition (g/litre)

Peptones 15.0; sodium chloride 5.0; yeast extract 3.0; sucrose 14.0; lactose 14.0; salicin 2.0; sodium thisulfate 5.0; ammonium iron(III) citrate 1.5; bile salt mixture 2.0; bromothymol blue 0.05; acidic fuchsin 0.08; agar-agar 13.5.

Preparation and Storage

Suspend 75 g in 1 litre of demin. water and let soak for 10 minutes.

Gently heat and bring to boil for a few seconds to dissolve the medium completely.

■ **Do not autoclave.**

If desired, add 15 mg novobiocin per litre to the cooled (50 °C) medium in form of a filter-sterilized solution. Pour plates.

pH: 7.7 ± 0.2 at 25 °C.

The plates are clear and blue-green.

Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25 °C.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.Experimental Procedure and Evaluation

Inoculate the culture medium with material taken from an enrichment culture by spreading thinly on the surface of the plates.

Incubation: 18-24 hours at 35 °C aerobically.

Colonies of the most important bacteria usually have the appearance described below. Colonies which are suspected to be pathogenic should be subjected to further tests to confirm their identity.

Appearance of Colonies	Microorganisms
Green, moist, flat, transparent	Shigella, Providencia
Blue-green, with or without a black centre	Salmonella, Paracolobactum, Proteus
Green to bluish, flat, irregular edge	Pseudomonas
Orange-red surrounded by a zone of precipitate	Coliform bacteria

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Literature

BISCIELLO, N.B. jr. a. SCHRADE, J.: Evaluation of Hektoen Enteric Agar for the detection of Salmonella in foods and feeds. - **Journ. of AOAC, 57**; 992-996 (1974).

HOBEN, D.A., ASHTON, D.H., a. PETERSEN, A.C.: Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved Salmonella isolation. - **Appl. Microbiol., 26**; 126-127 (1973).

KING, S. a. METZGER, W.J.: A new plating medium for the isolation of enteric pathogens. I. Hektoen Enteric Agar. - **Appl. Mikrobiol., 16**; 557-578 (1968).

KING, S. a. METZGER, W.J.: A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with SS- and EMB-Agar. - **Appl. Microbiol., 16**; 579-581 (1968).

TAYLOR, W.I., a. SCHELHART, D.: Isolation of Shigellae, VII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimen. - **Appl. Microbiol., 21**; 32-37 (1971).

Ordering Information

Product	Ordering No.	Pack size
HEKTOEN Enteric Agar	1.11681.0500	500 g
Novobiocin	491207	

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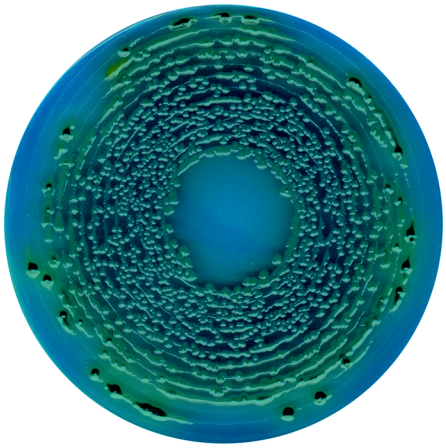
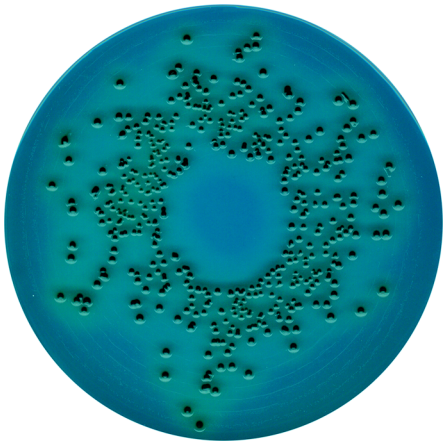
Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Rovery rate (%)	Colour	Colonies Black centre	Recipitate
Escherichia coli ATCC 25922	$< 10^5$	Not limited	orange-red	-	±
Enterobacter cloacae ATCC 13047	10^3-10^5	≥ 30	orange-red	-	±
Klebsiella pneumoniae ATCC 13883	10^3-10^5	≥ 30	orange-red	-	+
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 20	blue-green	+	-
Salmonella enteritidis ATCC 13076	10^3-10^5	≥ 20	blue-green	+	-
Shigella flexneri ATCC 12022	10^3-10^5	≥ 5	green to blue-green	-	-
Shigella sonnei ATCC 11060	10^3-10^5	≥ 20	green to blue-green	-	-
Proteus mirabilis ATCC 14273	10^3-10^5	≥ 30	green to blue-green	±	-
Enterococcus faecalis ATCC 11700	$> 10^5$	≤ 0.01			
Staphylococcus aureus ATCC 25923	$> 10^5$	≤ 0.01			



Proteus mirabilis ATCC 14273



Salmonella enteritidis ATCC 13076