

RTA.KK.361 Revision Date/Revision Number:-/0 Issue Date: 15.03.2017

SALMONELLA SHIGELLA AGAR / HEKTOEN ENTERIC AGAR

INTENDED USE:

A differential, selective medium recommended for the isolation of Salmonella and Shigella from stool, foods and clinical material.

Hektoen Enteric Agar is a moderately selective and differential medium for the isolation and cultivation of gram-negative enteric micro-organisms, especially for the isolation of Shigella and Salmonella species from fecal specimens.

PRINCIPLE AND INTERPRETATION:

SS Agar was originally developed as a selective medium for the isolation of Salmonella and Shigella species. It was also developed to aid in the differentiation of lactose and non-lactose-fermenters from clinical specimens, suspected foods, and other such samples. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms which ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red colonies. Lactose-nonfermenters form colorless colonies. The latter group contains the majority of the intestinal pathogens, including Salmonella and Shigella. The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

Bile salts render the medium selective, inhibiting Gram positive organisms and reducing growth of some gram-negative organisms other than Salmonella and Shigella. Lactose, sucrose and salicin are included for optimal differentiation by the color of the colonies and of the medium adjacent to the colonies. Salmonella and Shigella do not ferment these carbon compounds and thus do not cause a color change of the pH indicator system, while organisms fermenting one or several of these compounds to acids, e.g. E. coli, cause a color change to yellow or orange. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production by Salmonella. The pH indicator system consists of acid fuchsin and bromthymol blue.

COMPOSITION:

Ingredients	Gr/Liter
`Lab-Lemco' powder	5 gr
Peptone	5 gr
Lactose	10 gr
Bile salts	8,5 gr
Sodium citrate	10 gr
Sodium thiosuphate	8,5 gr
Ferric citrate	1 gr
Brilliant green	0,00033 gr
Neutral red	0,025 gr
Agar	15 gr

***Formula adjusted, standardized to suit performance parameters

pH 7.0 ± 0.2

Ingredients	Gr/Liter
Proteose peptone	12 gr
Yeast extract	3 gr
Lactose	12 gr
Sucrose	12 gr
Salicin	2 gr
Bile salts No.3	9 gr
Sodium chloride	5 gr
Sodium thiosulphate	5 gr
Ammonium ferric citrate	1,5 gr
Acid fuchsin	0,1 gr
Bromothymol blue	0,065 gr
Agar	14 gr

***Formula adjusted, standardized to suit performance parameters pH 7,5 ± 0.2

PRECAUTIONS:

For professional use only. Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

TEST PROCEDURE:

Faeces or rectal swabs may be plated directly or selective enrichment broths may be used prior to streaking out. Selenite Broth or Tetrathionate Broth may be used for salmonella enrichment.

1. Inoculate the poured, dried plates with a loopful of inoculum either from a suitable enrichment broth, from stool samples or rectal swabs.

2. Incubate the plates at 35-37°C for 18-24 hours.

QUALITY CONTROL:

1.Sterility Control:

Incubation 48 hours at 30-35°C and 72 hours at 20-25°C: NO GROWTH



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2.Phsical/Chemical Control

pH (Salmonella Shigella Agar): 7,0 \pm 0,2 / **pH** (Hektoen Enteric Agar) : 7,5 \pm 0,2 **Apperance**(Salmonella Shigella Agar): Red-Orange **Apperance**(Hektoen Enteric Agar): Green with yellowish cast

3.Microbiological Control: Cultural response on SS Agar / Hektoen Enteric Agar at 35± 2 C° after 24 and 48 hours incubation.

Microorganism	Inoculum	Results	
	(CFU)	Salmonella Shigella Agar	Hektoen Enteric Agar
Escherichia coli ATCC 25922	10-100	Partial inhibition	Orange colonies
Salmonella typhimurium ATCC 14028	10-100	Good	Black colonies
Enterococcus faecalis ATCC 29212	10-100	Partial inhibition	_
Shigella flexneri ATCC 12022	10-100	Colorless colonies	Green-Blue colonies
Proteus spp.	10-100		Black colonies

STORAGE CONDITIONS AND SHELF LIFE:

Store the prepared medium at 2-12°C. Use before expiry date on the label. Do not use beyond stated expiry date.

DISPOSAL:

Incubated prepared medium may contain active bacteria and micro-organisms. Do not open infected medium. Infected plate should be autoclaved, incinerated or opened and soaked in a chlorine-based disinfectant (liquid bleach) for 20 minutes prior to disposal.

PACKAGING:

Katalog Number: 03015 Packaging: Single wrap Content: 10 biplates/each package

REFERENCES:

SS Agar

1.Leifson, E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. and Bacteriol. 40:581-599.

2. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook, Vol. I, II & III. American Society for Microbiology, Washington, D.C. 4. MacFaddin, J.F. 1985. Media for Isolation, Cultivation, Identification, Maintenance of Bacteria, Vol. I. Williams & Wilkins, Baltimore, MD.

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1. King S. and Metzger W. I. (1968) Appl. Microbiol. 16. 577-561.

2. Taylor W. I. and Schelhaut D. (1971) Appl. Microbiol. 21. 32-37.

3. Hoben D. A., Ashton D. H. A. and Peterson A. C. (1973) Appl. Microbiol. 21. 126-129.

4. American Public Health Association (1992) Compendium of Methods for the Microbiological Examination of Foods 3rd Edition. APHA Inc. Washington DC.

