Endo HiCynth™ Agar

MCD029

Endo HiCynth™ Agar is a selective medium recommended for confirmation of the presumptive test for members of the coliform group from clinical and other samples.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.1*	10.000
Lactose	10.000
Dipotassium phosphate	3.500
Basic fuchsin	0.500
Sodium sulphite	2.500
Agar	15.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptone

Directions

Suspend 41.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. If the solidified culture medium is somewhat too red, then to remove the colour add a few drops (max. 1 ml/litre) of a freshly prepared 10% Sodium sulphite solution and boil.

Caution : Basic fuchsin is a potential carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin .

Principle And Interpretation

Endo Agar was developed by Endo to differentiate gram-negative bacteria on the basis of lactose fermentation, while inhibiting gram-positive bacteria (1). Inhibition of the later was achieved without the use of bile salts as was traditionally used. Endo was successful in inhibiting gram-positive bacteria on his medium by the incorporation of sodium sulphite and basic fuchsin. The resulting Endo Agar, also known as Fuchsin Sulphite and Infusion Agar, was used to isolate the typhoid bacilli. Many modifications of this media have been done over the years. Endo HiCynth™ Agar is prepared by using chemically defined peptone free from animal and vegetable peptones to avoid BSE/TSE risks associated with animal peptones. Endo Agar is recommended by APHA as an important medium in the microbiological examination of water and wastewater, dairy products and foods (2-4). Also is used to confirm the detection and enumeration of coliform bacteria following presumptive test of drinking water. It is also used for the detection and isolation of coliforms and fecal coliforms from milk, dairy products and food.

The medium contains HiCynth™ Peptone No.1 which provides nitrogen, carbon, long chain amino acids, vitamins and minerals required for bacterial growth. Sodium sulphite and basic fuchsin make this medium selective by suppressing gram-positive organisms. Coliforms produce pink colonies on fermention of lactose while lactose non-fermenters produce colourless colonies on the medium.

With *Escherichia coli* , this reaction is very pronounced as the fuchsin crystallizes, exhibiting a permanent greenish metallic luster (fuchsin luster) to the colonies. Medium should be stored away from light to avoid photo-oxidation.

Quality Control

Appearance

Light pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Orangish pink coloured, clear to slightly opalescent gel with fine precipitate forms in Petri plates.

Reaction
Reaction of 4.15% 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 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
Bacillus subtilis ATCC 6633	>=10^3	inhibited	0%	
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	>=50%	pink
Enterococcus faecalis ATCC 29212	50-100	none-poor	<=10%	pink, small
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=50%	pink to rose red with metallic sheen
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	>=50%	pink, mucoid
Proteus vulgaris ATCC 13315	50-100	good-luxuriant	>=50%	colourless to pale pink
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	>=50%	colourless, irregular
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%	colourless to pale pink
Shigella sonnei ATCC 25931	50-100	good-luxuriant	>=50%	colourless to pale pink
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	
Enterobacter cloacae ATCC 13047	50-100	good	40-50%	pink
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	>=50%	colourless
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant	>=50%	colourless
Shigella flexneri ATCC 12022	50-100	good-luxuriant	>=50%	colourless

Storage and Shelf Life
Store below 30°C in a tightly closed container and prepared medium at 2 - 8°C and away from light to avoid photooxidation. Use before expiry date on the label.

Reference
1. Endo S., 1904, Zentralbl. Bakteriol., Abt. 1, Orig.35:109-110.
2. Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A. W., (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd Ed., APHA, Washington, D.C.
3. Downes F. P. and Ito K.,(Eds.), 2001, Compendium of Methods for the Microbiological Examination of foods, 4th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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