

## Bile Esculin HiCynth™ Agar

MCD972

Bile Esculin HiCynth™ Agar is a differential medium recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

### Composition\*\*

Ingredients	Gms / Litre
HiCynth™ Peptone No.1*	40.000
HiCynth™ Peptone No.5*	6.000
Esculin	1.000
Ferric citrate	0.500
Agar	15.000
Synthetic detergent No.II	2.000
Final pH ( at 25°C)	6.6±0.2

\*\*Formula adjusted, standardized to suit performance parameters

\*Chemically defined peptones

### Directions

Suspend 64.5 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 and pour into sterile Petri plates. Alternatively it can be dispensed in tubes to form slants

### Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae*, *Klebsiella*, *Enterobacter*, *Serratia* from other *Enterobacteriaceae* genera (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5). Bile Esculin HiCynth™ Agar is prepared by completely replacing animal or vegetable based peptones with chemically defined peptone to avoid BSE/ TSE risks associated with animal peptones.

The medium is highly nutritious. HiCynth™ Peptone No.1 and HiCynth™ Peptone No.5 serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Synthetic detergent No. II inhibits most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also *Leuconostoc*, *Pediococcus*, *Lactococcus* species causing human infections give a positive bile esculin test. Inoculate and incubate the test sample in Todd Hewitt Broth (M313). After 24 hours incubation add two drops of the culture onto the surface of slant or plate media (3, 5).

### Quality Control

#### Appearance

Light yellow to brownish yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates or in tubes as slants.

**Reaction**

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 6.6±0.2

**pH**

6.40-6.80

**Cultural Response**

Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 hours.

**Cultural Response**

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<b>Cultural Response</b> <i>Enterococcus faecalis</i> ATCC 50-100 29212		luxuriant	>=50%	positive reaction,blackening of medium around the colony
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	>=50%	negative reaction
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	none-poor	<=10%	negative reaction

**Storage and Shelf Life**

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**

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