# Bile Esculin Agar ISO

Cat. 1031

For the isolation and presumptive identification of enterococci and for studies of fermentation of esculin by Yersinia

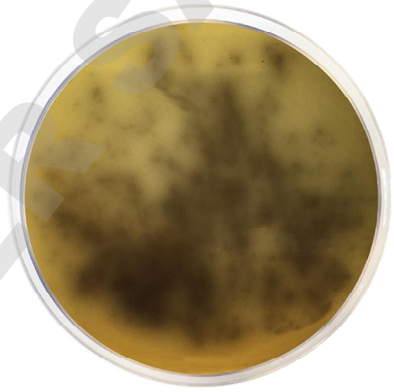
## Practical information

| Aplications | Categories |
|---|---|
| Confirmation | Yersinia enterocolitica |
| Selective isolation | Enterococci |

Industry: Clinical / Food

Regulations: ISO 10273

CE
IVD



## Principles and uses

Bile Esculin Agar is ideal for the isolation and differentiation of intestinal enterococci, based on Esculin hydrolysis in the presence of bile. It is also reccomended by ISO 10273 for fermentation studies of esculin by Yersinia. An esculin test shall be carried out to determine presumed pathogenicity since pathogenic Yersinia enterocolitica strains are esculin negative. This test for fermentation of esculin is equivalent to the test for fermentation of salicin.

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric citrate to form a dark brown or black colony. Bile Salts do not inhibit enterococci while other Gram positive bacteria are inhibited. Beef extract and peptone supply the nutrients essential for growth. Bacteriological agar is the solidifying agent.

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of Enterococci. The brown color (positive reaction) around the colonies appears after 18-24 hours of incubation at a temperature of 35±2 °C.

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including Escherichia coli, may already be dead when the analysis is carried out.

## Formula in g/L

| | | | |
|---|---|---|---|
| Bacteriological agar | 15 | Bile salts | 40 |
| Esculin | 1 | Beef extract | 3 |
| Meat peptone | 5 | Ferric citrate | 0,5 |

## Preparation

Suspend 64,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position.

## Instructions for use

» For clinical diagnosis, the type of sample is bacteria isolated from faeces.

- Inoculate on the surface making parallel striae with the handle or hyssop.
- Incubate in aerobic conditions at 35±2 ºC for 18-24 hours.
- Reading and interpretation of the results.

» For other uses not covered by the CE marking:

Isolation and presumptive identification of enterococci:
- Streak the slant surface of the agar.
- Incubate at a temperature of 35±2ºC for 18-24 horas
- Positive cultures are confirmed on KAA Confirmatory Agar (Cat. 1027) or KF Streptococcal Agar (Cat. 1034).

Confirmation of pathogenic Yersinia enterocolitica according to ISO 10273:
- From the colonies selected for confimation growth in CIN, streak the bacteria in a slanted tube of Bile Esculin Agar.
- Incubate at 30 ºC for 24±2 h.
- The appearance of a black halo around the colonies indicates a positive reaction.

## Quality control

| Solubility | Appareance | Color of the dehydrated medium | Color of the prepared medium | Final pH (25ºC) |
|---|---|---|---|---|
| w/o rests | Fine powder | Toasted | Litmus | 6,6±0,2 |

## Microbiological test

Incubation conditions: (35±2 ºC /18-24 h)
Yersininia enterocolitica according to ISO 10273 (30 °C/ 24 h).

| Microrganisms | Specification | Characteristic reaction |
|---|---|---|
| Streptococcus pyogenes ATCC 12344 | Inhibition | |
| Enterococcus faecalis ATCC 19433 | Good growth | Esculin Hidrolysis |
| Enterococcus faecium ATCC 19434 | Good growth | Esculin Hidrolysis |
| Staphylococcus aureus ATCC 25923 | Good growth | Esculin Hidrolysis (light) |
| Yersinia enterocolitica ATCC 27729 | Good growth | |
| Enterococcus faecalis ATCC 29212 | Good growth | Esculin Hidrolysis |
| Streptococcus pneumoniae ATCC 6305 | Inhibition | |

## Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

## Bibliography

Bact. Proceedings M33. 1969 Clin. Lab Forum July 1970.
Swan, A. 1954. The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (Group D streptococci). J. Clin Pathol 7:160 Facklam, R.R. and M.D. Moody 1 970 Presumptive identification of Group D streptococci, The bile esculin test. Appl. Microbiol 20:245.
Farmer J.J. III 1995 Enterobacteriaceae P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (eds) Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

ISO 10273. Microbiology of the food chain. Horizontal method for the detection of pathogenic Yersinia enterocolitica