

TECHNICAL DATA SHEET

Bile Esculin Azide Agar

Principle

Bile Esculin Azide Agar is a selective medium used for isolation and presumptive identification of fecal Streptococci. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7899-1:1984. The media is composed of tryptone, meat peptone, yeast extract, oxgall, sodium chloride, esculin, ferric ammonium citrate, sodium azide and agar. Tryptone and meat peptone provides nitrogen and other necessary minerals. Oxgall is selective agent inhibits gram positive bacteria other than enterococci. Sodium chloride maintains osmotic equilibrium. Ferric citrate is indicator for esculin hydrolysis. The esculin (glycoside) is source of carbon. The esculin hydrolyzing microorganisms hydrolyzes esculin to esculetin and dextrose. The esculetin reacts with ferric citrate to form brown or black color complex. Sodium azide inhibits most of the associated bacteria. Agar is solidifying agent. 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. The media can be supplemented with 50ml/l horse serum, to enhance the growth of Enterococci.

Use: For selective isolation and presumptive identification of faecal Streptococci.

Contents*

Ingredients	Gram/Litre
Tryptone	17.000
Meat peptone	3.000
Yeast extract	5.000
Oxgall	10.000
Sodium chloride	5.000
Ferric ammonium citrate	0.500
Esculin	1.000
Sodium azide	0.150
Agar	15.000
pH at 25°C	7.2 ±0.2

*** Formula adjusted for optimum performance and parameters**

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Directions: Dissolve 56.70 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 min (Overheating may causes darkening of medium), cool it to 42-45°C and dispense in desired (Test tubes to form butt and in petri plates for isolation and identification). Ensure complete solidification and inoculate test sample aseptically.

Specimens' types analyzed
Clinical samples etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Light brownish beige colored free flowing, homogeneous powder
Reaction of 5.67% solution	7.2 $\pm$ 0.2 at 25 °C
pH	7.00- 7.40
Gelling	Firm comparable with 1.5% agar gel
Color and clarity of ready medium	Amber colored opalescent gel with bluish tinge
Growth Promotion properties	Best at $\leq$ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at $\leq$ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

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Different Microbial Response: Cultural characteristics observed after incubation at $35\pm 2^{\circ}\text{C}$ for 18-24 hours. Inoculum 50-100 CFU.

Organism	ATCC	Growth	Recovery	Esculin hydrolysis
<i>Enterococcus faecalis</i>	14506	Luxuriant	$\geq 60\%$	Positive (Black color)
<i>Staphylococcus aureus</i>	25923	Good	$\geq 40\%$	Negative
<i>Proteus mirabilis</i>	12453	Good	$\geq 40\%$	Negative
<i>Escherichia coli</i>	8739	Inhibited	--	---

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
2. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. Facklam, R. R., and M. D. Moody. (1970). *Presumptive identification of group D streptococci: The bile-esculin test*. Appl. Microbiol. 20:245.
4. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover J. C., (2003), *Manual of Clinical Microbiology*, ASM, Washington, D.C. 8th Ed.

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