Tryptone Bile Glucuronic Agar (TBX Agar)

M1591

Intended use

Tryptone Bile Glucuronic Agar is selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs, animal feed, water and clinical samples.

Composition**

Ingredients	Gms / Litre
Bile salt mixture	1.500
Tryptone	20.000
X-ß-D-glucoronic acid	0.075
Dimethyl sulfoxide	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.6 grams in 1000 ml purified/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 in sterile Petri plates.

Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (3). Tryptone Bile Glucuronic Agar contains the enzyme ß -D- glucuronidase which differentiates most *E.coli* species from other coliforms.
E.coli absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (2).The enzyme ß-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the ß-D-glucuronide. *E.coli* colonies are blue green coloured (5,6). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

Type of specimen

Clinical samples - urine, blood, Food samples ; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.66% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 44°C for 18- 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii ATCC 8090	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	>=50%	blue-green
Enterococcus faecalis ATCC 29212 (00087)*	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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3.International Standard ISO 16649-2: 2018. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of presumptive *Escherichia coli*; Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

6. Killian M. and Bolow P 1976 Rapid diagnosis of Enetrobacteriacea I. Detection of bacterial glycosidases. Acta Rattol. Microbiol Scand Sct B 84245:251.

7. Ley A N, Bowers R J, Wolfe S 1988 Indocyl –B-D-glcuaoride, a novel chromogenic coli reagent for the detection and enumeration of Escherichia coli in environmental samples. Canadian Journal of Microbiology 34:690-693.

8. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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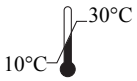
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg,Mumbai-86,MS,India

EC REP

CE Partner 4U ,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu