Also known as
TBX

Specification
Selective and differential solid medium for the detection and enumeration of ß-glucuronidasepositive *Escherichia coli* according to ISO standards.

Formula * in g/L
Tryptone..... 20.000
Bile salts No. 3..... 1.500
5-Bromo-4-chloro-3-indoxyl-ß-D-glucuronide..... 0.075
Agar..... 15.000

Final pH 7,2 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 36.5 g of the powder in 1 L of distilled water and heat to boiling with continuous stirring until total dissolution. Dispense into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description
Escherichia coli is the only coliform that possesses ß-D-glucuronidase and can be easily differentiated from other coliforms that do not show this enzymatic activity. There are some strains of *E. coli* (less than 3-4% of the total population) that are ß-D-glucuronidase negative.
E. coli absorbs the chromogenic substrate (X-ß-D-glucuronide) and the bacterial enzyme ß-D-glucuronidase splits the bond between the chromophoric X-fraction and the ß-D-glucuronide.
The free X-fraction dyes the *E. coli* cells and produces a blue-green colony.
The high content in bile salts of the medium inhibits the growth of accompanying Gram positive bacteria and the high incubation temperature (44ºC) inhibits Gram negative bacteria other than *E. coli*.

Technique
1. Direct inoculation (Pour plate technique)
Transfer 1 mL of test sample to a sterile Petri dish aseptically, and repeat the procedure with further dilutions. Inoculate two plates per dilution. Pour 15 mL of melted and cooled (44-47ºC) TBX Agar into each Petri dish. Mix carefully and allow the mixture to solidify. The time between the distribution of the inoculum and pouring the medium should not exceed 15 minutes.
Invert the inoculated plates and incubate them at 44±1ºC for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4h ±0,25 at 37±1ºC and then raise the incubation temperature to 44ºC. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45ºC.
2. Membrane incubation (Resuscitation technique)
No special membranes are recommended. Any sterile and non-inhibitive membrane made of cellulose acetate or mixed esters of cellulose, with 0.45 µm to 1.2 µm pore size and 85 mm diameter can be used.
2.1. Resuscitation
Aseptically place a membrane on the dried surface of each of two plates of Mineral-Modified-Glutamate Agar (MMGA) with care to avoid trapping air bubbles. Add 1 mL of the test sample to the centre of each membrane and spread the inoculum evenly over the whole membrane surface. Repeat the procedure for each dilution of the sample.
Leave the inoculated plates at room temperature for 15 minutes until the inoculum has soaked into the agar. Incubate the plates at 37±1ºC for 4 ± 0,25 hours.
2.2. Transfer to the selective medium
After the resuscitation period, transfer the membranes from the resuscitation medium to the plates of TBX Agar using sterile forceps, taking care to avoid trapping air bubbles beneath the membrane. Do not touch nor disturb the membrane surface. Incubate the plates for 20-24 hours at 44ºC (and not more than 45ºC).
3. Results
The ß-D-glucuronidase-positive Escherichia coli produces blue colonies (Blue-green). Some strains (3-4% of the total population) of E. coli lack the glucuronidase enzyme and produce colourless colonies. Some stressed cells of E. coli are unable to grow at 44ºC and produces no colonies.

Quality control

Incubation temperature: 44 ± 1° C **Incubation time:** 20-24 h

Inoculum: Practical range 100 ± 20 CFU; Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) / 10^3-10^4 CFU (Specificity) according to ISO 11133.

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC® 19433	Inhibited	Selectivity
Escherichia coli ATCC® 25922	Productivity > 0.50	Blue colonies
Escherichia coli ATCC® 8739	Productivity > 0.50	Blue colonies
E. coli NCTC® 13216	Productivity > 0.50	Blue colonies
C.freundii ATCC® 43864	Good	Colorless colonies

References

· DELISLE, G.L. & A. LEY (1989) Rapid detection of E. coli in urine samples by a new chromogenic ß-glucuronidase assay. J. Clin. Microbiol. 27:778-779

· ISO Standard 16649-1:2018. Microbiology of foods chain- Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* - Part 1: Colony count technique at 44ºC using membranes and 5-bromo-4-chloro-3-indolyl ß-D-glucoride.

ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

· OGDEN, I.D. & A.J. WATT (1991) An evaluation of fluorogenic and chromogenic assays for the direct enumeration of E. coli. Letters in Appl. Microbiol. 13:212-215.

· SCHWEIZERISCHES LEBENSMITTELBUCH (2005) Kap.56 Mikrobiologie, Bundesamt für Gesundheit. Direktionsbereich Verbraucherschutz. Bern.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).