

Specification

Selective and differential solid medium for the detection and enumeration of β -glucuronidase positive *Escherichia coli* according to ISO standards.

Presentation

| | Packaging Details | Shelf Life | Storage |
|--|--|------------|---------|
| 20 Prepared Plates 90 mm with: 21 \pm 2 ml | 1 box with 2 packs of 10 plates/pack. Single cellophane. | 3 months | 2-14 °C |

Composition

| | |
|---|--------|
| Composition (g/l): | |
| Tryptone..... | 20.000 |
| Bile salts no 3 | 1.500 |
| Agar..... | 15.000 |
| 5-Bromo-4-chloro-3-indoxyl- β -D-glucuronide..... | 0.075 |

Description /Technique

Description:

Escherichia coli is the only coliform that possesses β -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 β -D-glucuronidase negative.

E. coli absorbs the chromogenic substrate (X- β -D-glucuronide) and the bacterial enzyme β -D-glucuronidase splits the bond between the chromophoric X-fraction and the β -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 (44°C) inhibits Gram negative bacteria other than *E. coli*.

Technique: (according to ISO standar)

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 (44-47°C) 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 \pm 1°C for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4h \pm 0,25 at 37 \pm 1°C and then raise the incubation temperature to 44°C. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45°C.

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 μ m to 1.2 μ m 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 \pm 1°C for 4 \pm 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 44°C (and not more than 45°C).

3. Results

The β -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 44°C and produces no colonies.

Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7.2 ± 0.2 at 25°C

Microbiological control

Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity).

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosi. Incubate at 44 °C ± 1 °C for 20 - 24 h

Microorganism

Escherichia coli ATCC® 25922, WDCM 00013

Escherichia coli ATCC® 8739, WDCM 00012

Escherichia coli NCTC® 13216, WDCM 00202

Enterococcus faecalis ATCC® 19433, WDCM 00009

Citrobacter freundii ATCC® 43864, WDCM 00006 (37/44°C)

Growth

Good (≥ 50%) Blue colonie

Good (≥ 50%) Blue colonie

Good (≥ 50%) Blue colonie

Inhibited

Good - Colourless colonies

Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Bibliography

- DELISLE, G.L. & A. LEY (1989) Rapid detection of *E. coli* in urine samples by a new chromogenic β-glucuronidase assay. J. Clin. Microbiol. 27:778-779
- ISO Standard 16649-1:2018. Microbiology of foods chain- Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 1: Colony count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl β-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

Storage conditions: 2-14°C

Alternatively the plates may also be stored at the range of 2 - 25°C, with a proper performance of the medium, but some precautions must be taken into account:

- In the range of 2 - 8 °C avoid direct contact with surfaces that can freeze product.
- In the range of 15 - 25 °C, dehydration control must be taking in account.