

Specification

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

Presentation

20 Prepared Plates
90 mm
with: 21 ± 2 ml

Packaging Details

1 box with 2 packs of 10 plates/pack. Single cellophane.

Shelf Life

3 months

Storage

2-14°C

Composition

Composition (g/l):

Tryptone..... 20.000

Bile Salts 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 $44 \pm 1^\circ\text{C}$ for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of $4\text{h} \pm 0,25$ at $37 \pm 1^\circ\text{C}$ 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 $0.45\ \mu\text{m}$ to $1.2\ \mu\text{m}$ 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 $37 \pm 1^\circ\text{C}$ 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 controlSpiral Spreading: Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity).
Microbiological control according to ISO 11133:2014/ Adm 1:2018.

Aerobiosi. Incubate at 44°C± 1°C for 21h ± 3h.

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 (37°C ±1)**Growth**

Good (≥ 50%) Blue colonie

Good (≥ 50%) Blue colonie

Good (≥ 50%) Blue colonie

Inhibited

Good - Colourless colonies

Sterility Control

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

Check at 7 days after incubation in same conditions

Bibliography

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