TBX / Agar
(Tryptone Bile X - Glucuronide)

355-5309
356-4035

DEFINITION
Selective **chromogenic** medium for direct enumeration (without colonies confirmation) of *Escherichia coli* in products intended for human and animal consumption.

STANDARDS
FOOD MICROBIOLOGY
- **NF ISO 16649-1 (August 2001)**: Food Microbiology - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*: Part 1: Colony-count technique at 44℃ using membrane and 5-bromo-4-chloro-3-indolyl-β-D-glucuronate acid.

- **NF ISO 16649-2 (July 2001):** Food microbiology - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*: Part 2: Technique of colony count at 44℃ by means of 5-bromo-4-chloro-3-indolyl-β-D-acid.

- **XP ISO/TS 16649-3 (December 2005):** Food microbiology - Horizontal method for enumeration of β-glucuronidase-positive *Escherichia coli* : Part 3 : Most Probable Number Technique using 5-bromo-4 chloro-3-indolyl- β -D-glucuronate.

PRINCIPLE
The principle of the medium relies on detection of the β-D-glucuronidase of *Escherichia coli*.
The medium contains a complex (chromogen linked to a sugar) called 5-bromo-4-chloro-3-indolyl-β-glucuronide (BCIG) which is specific to β-D-glucuronidase.
Absorbed by the target micro-organism, the complex is hydrolysed by this enzyme. The sugar is consumed by the bacteria and the chromogen agent accumulates in this same cell, giving *E. coli* colonies a blue or blue-green color. Due to the conjugated action of high temperature and bile salts, this medium is selective with regard to Gram positive and interfering flora.

N.B.: Some E.coli strains:
- *are β-D-glucuronidase-negative (around 3-4%), and do not yield a blue color (e.g.* E. coli O157*),*
- *do not develop at the high temperature of 44℃ (e.g.* E.coli O157:H7*).*

Some serovars of Salmonella *and a few species of* Shigella *also possess the enzyme β-D-glucuronidase (< 1.5%).*

PRESENTATION
- **Ready to use**
  100 ml x 6 bottles **code 355-5309**

- **Dehydrated**
  500 g **code 356-4035**

STORAGE
- Ready-to-use: + 2℃ to 8℃ in the dark.
- Dehydrated: + 15℃ to 25℃, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.
- Medium prepared by user from dried product: 1 month at + 2℃ to 8℃ in the dark.

THEORETICAL FORMULA

Enzymatic casein digest	20 g
Bile salts N°3	1.5 g
BCIG	0.075 g
Agar	12 g
Distilled water	1,000 ml

Final $pH_{(25℃)}$ = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Mixer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Sterile spreaders
- Sterile membrane filters (Ø = 85 mm, 0.45 µm to 1.2 µm)
- Water-bath precise to ± 1℃
- Thermostatically-controlled incubator or incubation room, precise to ± 1℃
- Autoclave
- All usual laboratory equipment.

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PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use.

Dissolve 33.6 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained.
Heat gently swirling frequently, then bring to boiling point until completely dissolved.
Dispense, then sterilize in autoclave at 121℃ for 15 minutes.

Reconstitution ratio: 33.6 g/l
500 g of powder makes 14.8 liters of medium.

PROTOCOL

Preparation of samples

According to the standards applicable to the product concerned.

Inoculation (2 dishes/dilution)

With membrane (for products liable to contain severely stressed bacteria) (ISO 16649-1).

- Prepare Petri dishes with MMGA medium and with TBX medium (approximately 15 ml/dish).
- Under aseptic conditions, place the membrane on the dry surface of the MMGA agar.
- Transfer 1 ml of test specimen (liquid product) or 1 ml of stock suspension (other products) and/or 1 ml of its decimal dilutions to the center of the membrane.
- Spread the inoculum uniformly over the whole surface of the membrane, avoiding distributing it beyond the membrane.
- Leave the inoculated dishes in a horizontal position at room temperature for about 15 minutes, until the inoculum has been impregnated by the agar.
- Turn the dishes over and incubate at 37℃ (± 1℃) for 4 hours (± 1 h).
- After revitalization, transfer - using sterile tweezers - the membranes of MMGA medium to Petri dishes containing TBX medium.

Without membrane (stressed or non-stressed bacteria) (ISO 16649-2)

- Using sterile pipettes, transfer 1 ml of test specimen (liquid product) or 1 ml of stock suspension (other products) and/or 1 ml of its decimal dilutions to sterile Petri dishes.
- Quickly pour about 15 ml of medium, melted and cooled to 44℃- 47℃.
- Homogenize.
- Leave to cool on a cool, level surface.

Incubation

Turn the dishes over and incubate at 44℃ (± 1℃) for 21 hours (± 3 h).
NB: Incubation should not exceed 24 hours and 45°C.

Inoculation for MPN technique (1 dish / positive tube)

After incubation of MMG broths, using an inoculating loop, subculture each tube presenting yellow coloration on a dish of TBX medium, streaking to obtain isolated colonies incubate 20h to 24h at 44℃.

READING AND INTERPRETATION

Colony count (UFC)

After the incubation period, count any characteristic *E. coli* colonies, which are blue or blue-green. Other colonies are white/beige.

N.B.:

- *Select dishes containing fewer than 150 characteristic colonies and fewer than 300 colonies in all.*
- *Depending on the different methods of calculation, dishes containing no colonies may be selected.*

Expression of result/Calculations

Refer to standards ISO 16649-1, -2 and -3 and NF ISO 7218.

PRECAUTIONS

- For media which are ready-to-use or prepared in advance, avoid any prolonged overheating during fusion. To conserve an optimal quality the medium must not undergo more than 1 cycle of supercooling-gelification.

- The medium may present a frothy appearance after gelification in bottles. It nevertheless retains all its qualities when its appearance changes after melting and shaking.

- As development of colonies at the bottom of the Petri dish may interfere with reading (stains), the time lapse between depositing the inoculum in Petri dishes and addition of the medium does not exceed 15 minutes.

- Avoid trapping any air bubbles underneath the membrane when depositing it on the agar. If necessary, gently and carefully flatten the membrane using tweezers.

- If the presence of stressed *E.coli* is suspected when inoculating without a membrane, incubate 4 hours at 37℃ (± 1℃), then 21 hours (± 3 h) at 44℃ (± 1℃).

- Comply with Good Laboratory Practice.

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PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 18- 24 h incubation at 44°C	
	GROWTH	COLOR
Escherichia coli * ATCC 25922	+	Blue
Klebsiella pneumoniae NCTC 11228	+	White to beige-green
Enterococcus faecalis ATCC 19433	Inhibited	NA

*** Productivity yield ≥ 0.5 (Ref = TSA)**
NA = not applicable

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

TBX / *Escherichia coli* / Food products / Enumeration / BCIG / β-D-Glucuronidase / Chromogenic / Medium.

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