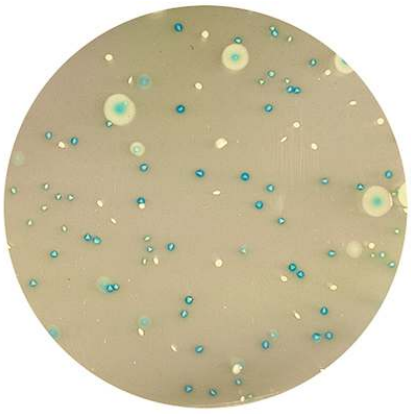
ChromArt

TRYPTONE BILE X-GLUC (TBX) AGAR

Dehydrated culture medium and ready-to use medium



TBX Agar: colonies of *E.coli* (blue-green) and *E.aerogenes* (white)

1 - INTENDED USE

For the enumeration of β-glucuronidase positive *Escherichia coli* in foods and animal feeding stuffs.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES, TUBES AND FLASKS

Tryptone (enzymatic digest of casein)	20.0 g
Bile Salts No. 3	1.5 g
Agar	14.0 g
5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (X-GLUC)^	75.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^: cyclohexammonium salt

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptone Bile X-Glucuronide (TBX) Agar is based on the formulation of Tryptone Bile Agar originally devised for the enumeration of *E. coli* in food materials.[1,2] TBX Agar is a modification of Tryptone Bile Agar developed on the basis of many studies on first fluorogenic and then chromogenic substrates for the detection of the enzyme β-glucuronidase directly on the isolation media[3-7] and contains the chromogenic compound 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (X-GLUC).
TBX Agar is prepared in accordance with ISO 16649 and meets the requirements therein.[8-10]
The enumeration of β-glucuronidase-positive *E. coli* in foodstuffs may be performed by 1) poured plate technique in TBX Agar[8], 2) colony-count technique using membranes overlaid on Minerals Modified Glutamate Agar, and subsequently transferred on TBX Agar[9], 3) most probable number technique using Minerals Modified Glutamate Medium and the subculture onto TBX Agar[10]. The last two techniques involve a resuscitation step and are recommended for the examination of foodstuffs likely to contain sub-lethally injured cells.
Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals. Bile salts n° 3, inhibit the development of Gram-positive bacteria, especially bacilli and faecal streptococci, while at the same time promoting *E. coli* growth. The medium contains X-GLUC for the detection of β-glucuronidase enzyme: within the *Enterobacteriaceae*, *E. coli* and a few strains of *Salmonella* and *Shigella* are able to split the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the ß-D-glucuronide; the released chromophore is coloured and builds up within the cells, causing *E. coli* colonies to be coloured blue-green.

4A- DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 35.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

4B- DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS AND TUBES)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, clear
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Tryptone Bile X-GLUC (TBX) Agar	Dehydrated medium	4021562	500 g (14 L)
Tryptone Bile X-GLUC (TBX) Agar	Ready-to-use plates	542156	2 x 10 plates ø 90 mm
Tryptone Bile X-GLUC (TBX) Agar	Ready-to-use plates	492156	3 x 10 plates ø 55 mm
TBX Agar	Ready-to-use tubes	5521562S	20 x 15 mL
TBX Agar	Ready-to-use flasks	5121562	6 x 100 mL
TBX Agar	Ready-to-use flasks	5121563	6 x 200 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile inoculation needles and pipettes, incubator and laboratory equipment as required, sterile Petri dishes, membrane filters, Erlenmeyer flasks, ancillary culture media and reagents.

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8 - SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Enumeration of *E. coli* by poured plate technique (ISO 16649-2)[8]

1. Transfer 1 mL of the test sample in duplicate, into two sterile Petri dishes if liquid, or 1 mL of the initial suspension (10^{-1}), in the case of other products. Repeat the procedure with further decimal dilutions if necessary.
2. Within 15 minutes, pour into each Petri dish about 15 mL of TBX Agar pre-cooled to 44-47°C.
3. Mix well the inoculum with the medium. Invert the inoculated dishes and incubate at 44°C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44°. Do not incubate over 45°C.

Enumeration of *E. coli* by membrane filtration technique (ISO 16649-1)[9]

1. Aseptically place a membrane onto the dried surface of an appropriate number of plates of Minerals Modified Glutamate Agar (MMGM REF 401737 supplemented with Agar) taking care to avoid trapping air bubbles beneath the membranes.
2. Transfer in the centre of the membrane 1 mL of the sample or 1 mL of the initial suspension and spread the inoculum on the surface of the membrane. Repeat the procedure with further decimal dilutions if necessary.
3. Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.
4. Leave the plates at room temperature for 15 minutes in order the medium adsorbs the liquid sample.
5. Incubate the plates for 4 h ± 0.25 h at 37 °C, with the membrane/agar surface uppermost.
6. After this resuscitation step transfer the membranes onto TBX Agar plates and incubate at 44°C for 18-24 hours. Do not incubate over 45°C.

Enumeration of *E. coli* by MPN technique (ISO 16649-3)[10]

1. Inoculate 3 or 5 tubes containing 10 mL of double-strength Minerals Modified Glutamate Medium (MMGM REF 401737) with 10 mL aliquots of the test sample, if liquid, or with 10 mL aliquots of the initial suspension in the case of other products.
2. Inoculate 3 or 5 tubes containing 10 mL of of single-strength MMGM with 1 mL aliquots of the test sample, if liquid or with 1 mL aliquots of the initial suspension in the case of other products.
3. Repeat the inoculation of the single strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution.
4. Incubate the tubes at 37°C for 24 ± 2 hours.
5. From each of the incubated tubes showing yellow colour subculture with a loop on a plate of TBX Agar by streaking to obtain isolated colonies and incubate at 44°C for 24 ± 2 hours.
6. Express the results as the Most Probable Number of *E. coli* on the basis of the presence of blue-green colonies on TBX plates.

10 - READING AND INTERPRETATION

After incubation, examine the TBX Agar plates for the presence of typical, blue or blue-green colonies indicating the presence of β-glucuronidase-positive *E. coli*.
Calculate the number of β-glucuronidase-positive *E. coli* by counting the typical colonies in each plate containing less than 150 typical CFU and less than 300 total (typical and non-typical) CFU.
Multiply the numbers of colonies by the dilution factor and express the result as the number of *E. coli* per gram of sample.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains			Incubation T°/ t - ATM	Expected results
E. coli	ATCC	25922	44°C / 18-24H / A	growth, blue-green colonies
C. freundii	ATCC	43864	44°C / 18-24H / A	growth, white to green-beige colonies
E. faecalis	ATCC	29212	44°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use TBX Agar are tested for productivity, specificity and selectivity, by comparing the results with Tryptic Soy Agar (TSA).
Productivity is assessed by a poured plate quantitative test with the target strains *E. coli* ATCC 25922, *E. coli* ATCC 8739 and *E. coli* NCTC 13216. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 44°C for 18 hours. The colonies are enumerated on TBX Agar and on TSA and the productivity ratio (Pr: CFU_{TBX}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (blue-green colonies) the results are considered acceptable and conform to the specifications.
Specificity is tested by semi-quantitative ecometric technique with the following non-target strains: *C. freundii* ATCC 43864 and *P. aeruginosa* ATCC 27853. After incubation at 44°C for 24 hours, the amount of growth and the colony characteristics are evaluated: non-target strains exhibit growth with white to green-beige colonies.
Selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. faecalis* ATCC 29212. The growth of the non-target strain is totally inhibited after incubation at 44°C for 24 hours.

13 - LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[11]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[12]
- Some strains of *E. coli* may grow poorly or not at all in media incubated at 44 °C. Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected by the methods reported above taken from ISO Standards.[10]

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use tubes/flasks are subject to terminal sterilization by autoclaving
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready-to-use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at +2°C /+8°C away from direct light. If properly stored, the tubes/flasks may be used up to the expiration date. Do not use the tubes/flasks beyond this date. Tubes/flasks s from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes/flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 16649-1 the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up for up to four weeks.[9] Immediately before use, dry the agar plates carefully.

16 - REFERENCES

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2. Anderson JM, Baird-Parker AC A rapid and direct plate method for enumerating Escherichia coli biotype I in food. J Appl Bacteriol 1975;39:111-7.
3. Kilian M, Bulow P. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol. Scand. Sect. B. 1976; 84:245–251.
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6. Restaino L., Frampton EW, Lyon RH. Use of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC) for enumerating Escherichia coli in 24 h from ground beef. J Food Prot 1990; 53:508–510.
7. Ogden ID, Watt AJ. An evaluation of fluorogenic and chromogenic assays for the direct enumeration of Escherichia coli. Lett Appl Microbiol 1991; 13:212–215.
8. ISO 16649-2:2001 Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
9. ISO 16649-1:2018. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
10. ISO 16649-3:2016. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide.
11. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
12. Robison BJ.Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl Environ Microbiol 1984; 48:285-288

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history

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