

ChromArt

CHROMOGENIC COLIFORM AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium


 Chromogenic Coliform Agar ISO Formulation.
Escherichia coli : blue colonies; *Citrobacter freundii*: red colonies

1 - INTENDED USE

 Chromogenic medium for the enumeration of *Escherichia coli* and coliform bacteria with membrane filtration technique on waters with low bacterial background, according to ISO 9308-1.

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

Enzymatic digest of casein	1.00 g
Yeast extract	2.00 g
Sodium chloride	5.00 g
Sodium dihydrogen phosphate x 2H ₂ O	2.20 g
Di-sodium hydrogen phosphate	2.70 g
Sodium pyruvate	1.00 g
Sorbitol	1.00 g
Tryptophane	1.00 g
Tergitol® 7	0.15 g
Salmon-β-D-galactoside	0.20 g
X-β-glucuronide CHX salt	0.10 g
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0.10 g
Agar	10.60 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

 Faecal pollution is the major cause of waterborne disease, since most of the pathogens associated with transmission reside in human and warm-blooded animal faeces. Examination of water samples for the presence of *E. coli* and coliform bacteria provides an indication of such pollution.

 Chromogenic Coliform Agar ISO Formulation is a selective and differential medium for the simultaneous detection and enumeration of β-glucuronidase-positive *E. coli* and β-D-galactosidase positive coliform bacteria from water samples with low bacterial numbers, such as drinking water, disinfected pool water, or finished water from drinking water treatment plant, according to ISO 9308-1.^{1,2}

Enzymatic digest of casein provides nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance while phosphates act as buffer of the medium. Sorbitol and sodium pyruvate stimulate a quick bacterial growth and aid in resuscitation of stressed cells. Tergitol 7 is a surfactant that acts as a selective agent, moderately inhibiting the growth of Gram-positive bacteria.

 Detection of coliform bacteria is based on the ability of β-D-galactosidase to cleave the substrate salmon-β-D-galactoside with the formation of salmon red colonies. Enumeration of *E. coli* is based on the detection of two enzymatic activities, β-D-glucuronidase and β-D-galactosidase, which cleave the chromogenic substrates salmon-β-D-galactoside and X-β-glucuronide, with the formation of dark blue colonies. The hydrolysis of X-GAL is enhanced by IPTG, a lactose operon inducer.

4A- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 27.1 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved. Do not autoclave, do not overheat. Cool to 47-50°C, mix well and dispense in sterile Petri dishes to a depth of at least 4 mm. Some turbidity may occur after boiling; it normally disappears when the temperature decreases to 47-50°C and doesn't affect the medium performances.

4B DIRECTIONS FOR BOTTLED MEDIUM PREPARATION

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
Prepared plates and flasks appearance	pale yellow, limpid or slightly opalescent
Final pH of complete medium (at 20-25°C)	6.8 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Chromogenic Coliform Agar ISO Formulation	Dehydrated medium	4012972	500 g (18.4 L)
Chromogenic Coliform Agar ISO Formulation	Ready-to-use plates	491297	3 x 10 plates ø 55 mm
Chromogenic Coliform Agar ISO Formulation	Ready-to-use flasks	5112972	6 x 100 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops, swabs, pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, membrane filters, ancillary culture media and reagents.



8 - SPECIMENS

Water samples with low bacterial numbers, such as drinking water, disinfected pool water, or finished water from drinking water treatment plant. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards and norms.

9 - TEST PROCEDURE

Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.

After filtration place the membrane filter on medium surface, ensuring that no air is trapped underneath, invert Petri dish, and incubate at 36 ± 2 °C for 21-24 h.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and count:

All pink to red colonies (positive for β-D-galactosidase reaction) as presumptive coliform bacteria other than *E. coli*.

All dark-blue to violet colonies (positive for β-D-galactosidase and β-D-glucuronidase reactions) as *E. coli*.

To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp, the presumptive colonies shall be confirmed by a negative oxidase reaction (Oxidase test Strips, cat. N° 191040ST)

Perform the oxidase test preferentially on all, or at least 10 selected pink to red colonies (coliform bacteria, different from *E. coli*): the test should be negative.

The count of total coliform bacteria is the sum of all oxidase negative pink to red colonies plus all dark-blue to violet colonies.

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
<i>E. coli</i> ATCC 25922	34-38°C/21-24H/A	growth, blue colonies
<i>E. aerogenes</i> ATCC 13048	34-38°C/21-24H/A	growth, pink colonies
<i>P. aeruginosa</i> ATCC 10145	34-38°C/21-24H/A	growth, colourless colonies
<i>E. faecalis</i> ATCC 19433	34-38°C/21-24H/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 Chromogenic Coliform Agar ISO Formulation are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by a quantitative test with the target strains *E. coli* ATCC 25922, *E. coli* ATCC 8739, *E. aerogenes* ATCC 13048, *C. freundii* ATCC 43864; the membrane filters on the medium are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 35-37°C for 21-24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio ($Pr = CF_{TB}/CF_{RB}$) is calculated. If $Pr \geq 0,7$ and if the colonies' morphology and colour are typical, the results are considered acceptable and conform to the specifications.

Specificity is tested by semi-quantitative technique with *P. aeruginosa* ATCC 10145. After incubation at 35-37°C for 21-24 hours *P. aeruginosa* grows with colourless colonies.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target Gram positive strain *E. faecalis* ATCC 19433. The growth of non-target strain is totally inhibited.

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%).³⁻⁵
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.^{1,4} Consequently, these strains, being positive for β-galactosidase, will grow with red-pink colonies and be counted as coliforms.¹
- In addition to expressing β-D-glucuronidase, *E. coli* is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.¹
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for Laboratory use 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.
- 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 inoculated plates 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 Sheet 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

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 (plates/flasks) and the applied storage conditions (temperature and packaging). According to ISO 9301-1 the plates can be stored at 5 ± 3°C in the dark and protected against evaporation for at least one month.

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 flasks

Upon receipt, store flasks in their original pack at +2°C /+8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks 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 flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

16 - REFERENCES

1. ISO 9308-1:2014 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.
2. ISO 9308-1:2014/AMD 1:2016 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora — Amendment 1
3. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
4. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
5. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.

TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/06

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

