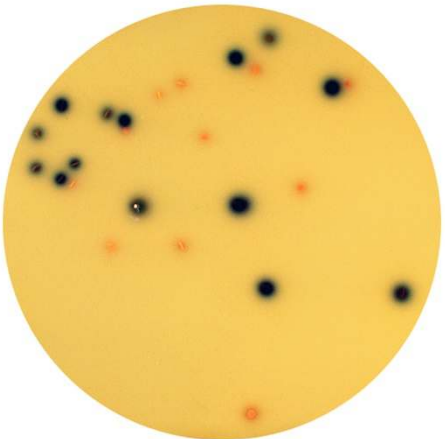
ChromArt

CHROMOGENIC COLIFORM AGAR

Dehydrated and ready-to-use culture medium



Chromogenic Coliform Agar: *E. coli* blue-grey colonies;
E. aerogens salmon colonies

1 - INTENDED USE

Chromogenic medium for the simultaneous enumeration of *Escherichia coli* and coliform bacteria.

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

Tryptose	10.00 g
Tryptophane	0.10 g
Peptocomplex	5.00 g
Yeast Extract	3.00 g
Sodium Chloride	5.00 g
Bile Salts n.3	1.50 g
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0.10 g
X-β-glucuronide CHX salt	0.06 g
Salmon-β-D-galactoside	0.15 g
Agar	13.00 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 is reported in the review of water analysis media ISSN:1125-2464[1] and in the APAT-IRSA[2] review for the simultaneous detection and enumeration of β-glucuronidase-positive *E. coli* and β-D-galactosidase positive coliform bacteria from water samples.

Peptocomplex and tryptose provide 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. Bile salts n° 3 act as a selective agent, 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-grey colonies. The hydrolysis of X-GAL in enhanced by IPTG, a lactose operon inducer. The tryptophane in the medium, allows the indole test to be performed directly on colonies with the addition of Kovacs' reagent, for confirmation of *E. coli*.

4A - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 37.9 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and dispense in sterile Petri dishes.

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

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	Dehydrated medium	4012992	500 g (13.2 L)
Chromogenic Coliform Agar	Ready-to-use plates	491299	3 x 10 plates, Ø 55 mm
Chromogenic Coliform Agar	Ready-to-use plates	541299	2 x 10 plates, Ø 90 mm
Chromogenic Coliform Agar	Ready-to-use flasks	5112992	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 and other materials of sanitary importance. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards and norms.

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9 - TEST PROCEDURE

Perform simultaneous enumeration of coliforms and *Escherichia coli*, applying normal laboratory methods with inoculation by pour plate method either by surface spread technique or by membrane filtration.
Incubate for 18-24 hours at 37°C.
The APAT-IRSA[2] review of microbiological methods for the determination of *E. coli* in water reports the following procedure:
Filter an aliquot of the sample or a volume of its dilutions 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. After filtration place the membrane filter on medium surface, ensuring that no air is trapped underneath, invert Petri dish, and incubate at 36°C ± 1°C for 18-24 hours.

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 blue-grey, indole-positive 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)
The count of total coliform bacteria is the sum of all oxidase negative pink to red colonies plus all blue-grey 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
E. coli ATCC 25922	35-37°C/18-24H/A	growth, blue-grey colonies
E. aerogenes ATCC 13048	34-38°C/18-24H/A	growth, salmon colonies
E. faecalis ATCC 19433	34-38°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 Chromogenic Coliform Agar are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch and Tryptic Soy Agar (TSA).
Productivity is tested by a quantitative test with the target strains *E. coli* ATCC 25922 and *E. aerogenes* ATCC 13048; the membrane filters on the medium are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 37°C for 24 hours.
The colonies are enumerated on Test Batch (TB) and TSA and the productivity ratio ($Pr=CFU_{TB}/CFU_{TSA}$) is calculated. If Pr is ≥ 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 *E. coli* ATCC 13762 *C. freundii* ATCC 8090, S.Enteritidis ATCC 13076, P.*vulgaris* ATCC 9484 and *P. aeruginosa* ATCC 10145. After incubation at 37°C for 24 hours *E coli* grows with grey-blu colonies, *C. freundii* grows with salmon colonies S. Enteritis*, P,vulgaris and 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 strains *E. faecalis* ATCC 19433 and *S.aureus* ATCC 25923. The growth of both non-target strains 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.[6] Consequently, these strains, being positive for β-galactosidase, will grow with red-pink colonies and be counted as coliforms.
- 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.
- 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).

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. APAT-IRSA Metodi analitici per le acque, vol 3 n° 7000 – Metodi per la determinazione di microrganismi indicatori di inquinamento e di patogeni. n° 7030, *2003, Escherichia coli.*
2. Bonadonna L. *Escherichia coli* nelle acque significato sanitario e metodologie di analisi. ISSN:1125-2464, 2001
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.
6. 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	For single use only	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2024/10

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