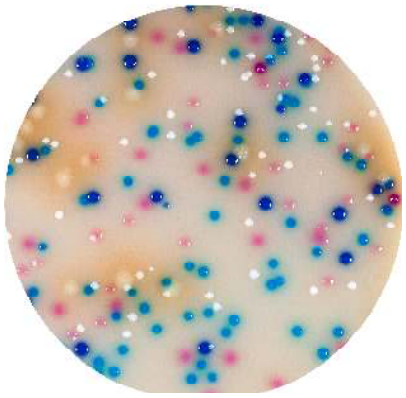
INSTRUCTIONS FOR USE

ChromArt

CHROMOGENIC URINE AGAR IV

Ready-to-use plates



Mixed culture of UTI pathogens: *E.coli* (pink-magenta red colonies), *K.pneumoniae* (dark blue colonies), *Enterococcus* sp. (turquoise blue colonies), *S.aureus* (white colonies), *Proteus* sp. (light brown with brown halo).

1 - INTENDED USE

In vitro diagnostic. Chromogenic culture medium for isolation, enumeration and presumptive rapid identification of microorganisms from urine.

2 - COMPOSITION - TYPICAL FORMULA *

Peptones and growth factors	24.0 g
Chromogenic mix	0.4 g
Opacifier compounds	10.0 g
Agar	15.0 g
Horse Serum	20.0 mL
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the last 25 years, chromogenic culture media have found widespread application in diagnostic clinical microbiology. Biolife proposed a chromogenic and fluorogenic medium for the diagnosis of urinary tract infections in 1997 named Chromogenic Urine Agar, in which the bacterial differentiation was obtained by the detection of β-glucuronidase, β-glucosidase and β-xylosidase. This first medium was subjected to incremental improvements over the years and new generations of media have been developed. The last one proposed by Biolife for urinary bacteriology is Chromogenic Urine Agar IV, a diagnostic medium intended for the isolation, enumeration and presumptive rapid identification of urinary tract pathogens such as *E.coli*, *Enterobacter-Klebsiella-Serratia*, *Proteus-Morganella-Providencia*, Enterococci, Staphylococci, yeasts.

The differentiation between the microbial species or genus is achieved by:

- A chromogenic substrate for β-galactosidase (GAL), which is cleaved by *E.coli* with the release of an insoluble pink-red chromophore.
- A chromogenic glucopyranoside derivative for β-glucosidase (GLU), which is cleaved by Enterococci with the formation of an insoluble blue-green dye.
- Tryptophan for the detection of tryptophan deaminase (TDA) and for performing rapid indole test.

Bacteria that produce both the enzymes (GAL and GLU), such as *Klebsiella/Enterobacter/Serratia* (KES) group, give dark blue or purple colonies. Tryptophan is present in the medium to detect members of the *Proteus* group, which generate a diffuse brown coloration as a result of tryptophan deaminase activity.

E.coli may be confirmed by indole spot test by adding a drop of Kovacs' Reagent to isolated colonies.

Main characteristics and advantages of CUA IV are: very good productivity obtained with selected and standardized peptones, optimized agar concentration to inhibit the swarming of Proteus spp., enhanced visual differentiation of the colonies due to strong chromatic reactions, grey, opaque contrasting background and specific enzymatic reactions for presumptive identification of both gram-positive and gram-negative pathogens.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	greyish, opaque
Final pH at 20-25°C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Urine Agar IV	Ready-to-use plates	549810G	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Chromogenic Urine Agar IV (CUA IV) is intended for the microbiological processing of clinical specimens such as urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1,2]

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Mix the urine gently to avoid foaming. Dip the end of a sterile calibrated loop (e.g. 1µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on

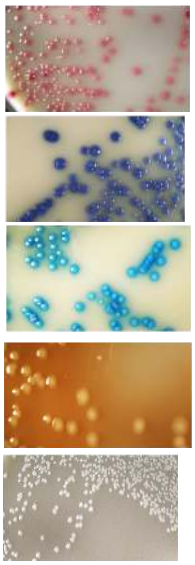
Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

the shank. Use this to inoculate CUA IV plate from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back-and-forth fashion. The inoculum of urine is spread over the entire agar surface to simplify counting of colonies after growth.
Incubate at 35-37°C in air for 24 to 48 hours.
Although most urinary tract pathogens grow readily, slowly growing pathogens and those inhibited by the presence of antimicrobials in the patient's specimen may not appear after overnight incubation (16 h). Perform leukocyte esterase and nitrite tests to determine which cultures get incubated for a full 48 hours. Urine cultures that are negative after overnight incubation but had one or both positive enzyme tests should be incubated for an additional day or re-inoculated on a blood agar plate.[1]

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth, count the number of colonies (CFU) on the plate and record the specific morphological, chromatic characteristics of the colonies. If a 1µL loop is used, one colony equals 1000 CFU/mL, if a 10µL loop is used, one colony equals 100 CFU/mL. Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of $\geq 10^5$ CFU/mL are indicative of an infection and counts below this usually indicate contamination.[2]
In specific patient groups, counts between 10^5 CFU/mL and 10^2 CFU/mL may be significant; a pure isolate with counts between 10^4 and 10^5 CFU/mL should be evaluated based on clinical information or confirmed by repeat culture.[2] For urine collected by suprapubic bladder puncture any CFU detected indicates an infection.[2]
Consult appropriate references for complete interpretation criteria of the microbial count.[1,2]

Typical colonial morphology and colours interpretation on Chromogenic Urine Agar IV are as follows:



Escherichia coli: pink colonies (β-galactosidase positive, β-glucosidase negative)
Indole test positive: *E.coli*
Indole test negative: proceed to the identification with conventional methods.

Klebsiella – Enterobacter - Serratia (KES): blue/blue-violet colonies:
(β-galactosidase positive, β-glucosidase positive)
Microscopic examination: gram negative bacilli
For genus/species identification, proceed with conventional identification methods.

Enterococcus spp.: green to turquoise blue colonies (β-galactosidase neg., β-glucosidase pos.)
Microscopic examination: gram positive cocci.

Proteus-Morganella-Providencia: (brown colonies: tryptophan deaminase positive, β-galactosidase negative, β-glucosidase negative)
Indole test negative: *Proteus mirabilis*, to be confirmed with oxidase test which is negative.
Indole test positive: *Providencia* or *Morganella* or *Proteus* spp. indole + (proceed to the identification)

Staphylococci and yeasts: white colonies (β-galactosidase negative, β-glucosidase negative)
Microscopic examination: gram positive cocci or yeasts
Proceed to the identification with conventional methods.

10 - 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.[3]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
E.coli	ATCC	25922	35-37°C / 18-24H / A	good growth, pink colonies
E.faecalis	ATCC	29212	35-37°C / 18-24H / A	good growth, green colonies
S.aureus	ATCC	25923	35-37°C / 18-24H / A	good growth, white colonies
K.pneumoniae	ATCC	27736	35-37°C / 18-24H / A	good growth, dark blue colonies
P.mirabilis	ATCC	12453	35-37°C / 18-24H / A	good growth, bluish colonies not swarmed

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Chromogenic Urine Agar IV and of the raw material used for the production of prepared plates (dehydrated Chromogenic Urine Agar IV REF 409810G) are tested for productivity by comparing the results with a previously approved Reference Batch and with Tryptic Soy Agar.
Productivity is tested by a quantitative test with the target strain *S.aureus* ATCC 25922; Chromogenic Urine Agar IV Agar and TSA plates are inoculated with decimal dilutions in saline of a colonies suspension and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on CUA IV and TSA plates and the productivity ratio is calculated ($Pr = CFU_{CUA\ IV}/CFU_{TSA}$). If *Pr* is ≥ 0,5 and if the colonies morphology and colour are typical (white colonies) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains: *E.coli* ATCC 25922, *E.coli* ATCC 8739, *P.mirabilis* ATCC 12453 *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736, *C.freundii* ATCC 8090 *C.diversus* ATCC 40738, *S.saprophyticus* ATCC 15305, *E.faecalis* ATCC 29212, *S.epidermidis* ATCC 12228, *C.albicans* ATCC 10231. After incubation, the colours of the colonies and the amount of growth are evaluated and recorded. All strains show a good growth with typical colours, according the specifications.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

12 - LIMITATIONS OF THE METHOD

- Gram staining is recommended to confirm any doubtful colour reactions.
- *Citrobacter* spp. may be presumptively identified as *E.coli* because some strains are β-galactosidase positive and β-glucosidase negative. The use of a spot indole test successfully eliminates some of these false positives[4]. The use of susceptibility data or the detection of pyrrolidonyl aminopeptidase (PYR test) may facilitate the differentiation of pink colonies of *Citrobacter* spp. from *E.coli*.[5]
- Between the *Proteus-Morganella-Providencia* group, *P.mirabilis* is indole negative and can be easily recognised.
- Biochemical identification is needed for genus/species identification within *Klebsiella, Enterobacter, Serratia* group.
- A pyrrolidonyl aminopeptidase (PYR test) might be necessary to differentiate enterococci from *S.agalactiae*.
- *S.saprophyticus* produces small blue colonies and *S.xylosus* produces small pink colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, of indole test or Gram morphology, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a semi-quantitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each 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.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°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).

15 - REFERENCES

1. McElvania E, Singh K. Specimen Collection, Transport and Processing:Bacteriology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Public Health England UK Standards for Microbiology Investigations. Investigation of urine. Bacteriology, B 41, 2019
3. Guidelines for assuring quality of medical microbiological culture media. The Australian Society for Microbiology, 2nd edition, 2012.
4. Perry JD, Butterworth LA, Nicholson A, Appleby MR, Orr KE. J Clin Pathol 2003; 56(7): 528–531
5. Fallon D, Andrews N, Frodsham D, Gee B, Howe S, Iliffe A, Nye KJ, Warren RE. J Clin Pathol 2002; 55(7): 524–529

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 3	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 4	Removal of obsolete classification	2023/04

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it