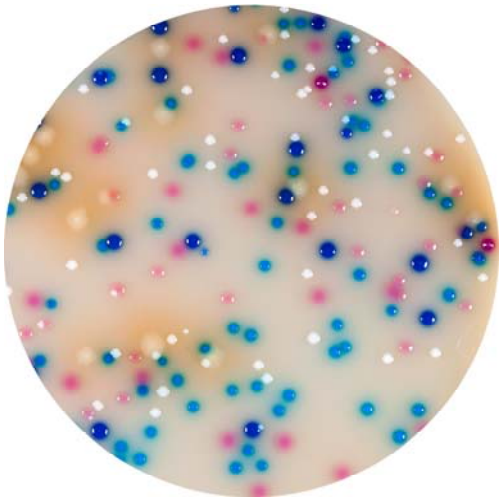
ChromArt

CHROMOGENIC URINE AGAR IV

Cultural response



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).

Typical formulas

Dehydrated medium (g/L)
ChromArt CHROMOGENIC URINE AGAR IV

Peptones and growth factors	24,000
Chromogenic mix	0,400
Opacifier compounds	10,000
Agar	15,000

Ready to use plates (g/L)
ChromArt CHROMOGENIC URINE AGAR IV

Peptones and growth factors	24,000
Chromogenic mix	0,400
Opacifier compounds	10,000
Horse Serum (ml/L)	20,00
Agar	15,000

Intended use

Improved non-selective differential medium with opaque grey background for presumptively identifying bacterial isolates from primary clinical specimens and for the enumeration of UTI pathogens.

Principle of the method and explanation

Chromogenic Urine Agar IV is a diagnostic medium useful for the isolation, counting and direct presumptive rapid identification of urinary tract pathogens: *E.coli, Enterobacter-Klebsiella-Serratia* – (KES), *Proteus-Morganella-Providencia*, Enterococci, Staphylococci, yeasts.

Main characteristics and advantages:

- 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 and to opaque contrasting background.
- Specific enzymatic reactions for presumptive identification of both gram positive and gram negative pathogens

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

- A chromogenic substrate for β-galactosidase (GAL) which is split with the liberation of an insoluble pink-red dye.
- A chromogenic glucopyranoside derivative which is split by β-glucosidase (GLU) with the formation of an insoluble blue-green dye.
- Tryptophan for the detection of tryptophan deaminase (TDA) and for indole test.

Strains that produce β-glucosidase, such as enterococci and the *Klebsiella/ Enterobacter/ Serratia* (KES) group, form colonies that generate a green/blue coloration as a result of hydrolysis of the indoxylic substrate. Strains of *Escherichia coli* appear as pink colonies because of β-galactosidase production. Tryptophan is also present in the medium to detect members of the *Proteae* group, which generate a diffuse brown coloration as a result of tryptophan deaminase production.
E.coli may be confirmed by indole spot test by adding a drop pf Kovacs' Reagent to isolated colonies.

Directions for dehydrated medium

Suspend 49,4 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. The prepared plates shall be used within ten days from the date of preparation.
For prolonged storage of the pre-poured plates add horse serum: cool the autoclaved medium to 45-50°C and add under aseptic conditions, 20 mL/L of Horse Serum. Mix well and distribute into sterile petri dishes.

Physical characteristics

Dehydrated medium appearance: grey, fine, homogeneous, free-flowing powder.
Prepared medium appearance: grey opaque. Final pH: 7,2 ± 0,2

Specimens

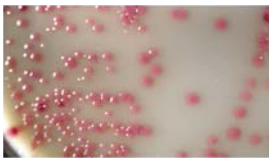
Specimens consist of urine samples.

Technique

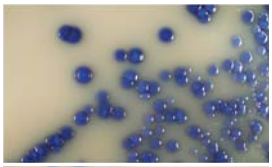
Chromogenic Urine Agar IV (CUA IV) can be used according to the usual laboratory practices for urine bacterial count, by spreading the specimen on the agar surface and incubating at 37°C for 18-24 hours.

Interpretation of the results

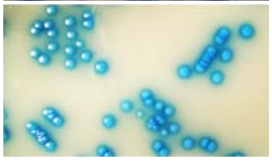
The cultivated colonies can be identified with the following scheme:



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.: 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*.
Indole test positive: *Providencia* or *Morganella* or *Proteus* spp. indole + (proceed to the identification with conventional methods,

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.

Limitations of the method

- The identification obtained with the medium should be considered as a presumptive identification. It must be confirmed with biochemical, immunological or other appropriate identification test. Biochemical tests are necessary for species identification of microorganisms producing colourless colonies. Gram staining is recommended to confirm any doubtful colour reactions.
- It is reported that some strains of the bacterial genus reported above have abnormal biochemical patterns.
- *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 (1). 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* (2).
- Between the *Proteus-Morganella-Providencia* group, *P.mirabilis* is indole negative and can be easy recognised
- Biochemical identification is needed for genus/species identification within *Klebsiella* , *Enterobacter*, *Serratia*, *Citrobacter* (KESC group).

- A pyrrolidonyl aminopeptidase (PYR test) might be necessary to differentiate enterococci from *S.agalactiae*
- *S.saprophyticus* and *S.xylosus* produces small pink colonies.
- Interpretation of the test results should be done considering the patient's history, the source of the specimen, colonial and microscopic reading and the results of other tests performed.

Quality control: microbiological characteristics

The user is responsible for performing the quality control according to national regulations.
The following strains are used by Biolife for the quality control of dehydrated medium and given as an example for the user's quality control.

Test Strains			Incubation T° / t / Atm.	Growth characteristics
E. coli	ATCC	25922	37°C - 24H-A	Good growth, pink colonies, indole positive
E. coli	ATCC	8739	37°C - 24H-A	Good growth, pink colonies indole positive
K. pneumoniae	ATCC	27736	37°C - 24H-A	Good growth, pale violet colonies
E. cloacae	ATCC	13047	37°C - 24H-A	Good growth, grey blue colonies
E. aerogenes	ATCC	13048	37°C - 24H-A	Good growth, pale blue colonies
C. freundii	ATCC	8090	37°C - 24H-A	Good growth, pale blue-pink colonies
C. diversus	ATCC	40738	37°C - 24H-A	Good growth, grey-pale blue
P. mirabilis	ATCC	10005	37°C - 24H-A	Good growth, brown-orange colonies
S. aureus	ATCC	25923	37°C - 24H-A	Good growth, white colonies
S. saprophyticus	ATCC	15305	37°C - 24H-A	Good growth, small pink colonies
E. faecalis	ATCC	19433	37°C - 24H-A	Good growth, turquoise blue/green colonies
S. epidermidis	ATCC	12228	37°C - 24H-A	Good growth, white colonies

Notes: A: aerobic incubation

Precautions

- Bring the plates to room temperature before the use
- For *in vitro* diagnostic use only; for professional use only.
- Dispose used plates as well as any other contaminated materials following procedures for infectious or potentially infectious products, in accordance with any applicable regulations.

Storage

Dehydrated medium: keep tightly closed, away from bright light, at 2°C to 8 °C.
Store the ready to use plates in their original box at 2°C to 8 °C.
When stored as directed the products remain stable until the expiry date shown on the label. Do not use beyond stated expiry date.

References

1. J. D. Perry, L. A. Butterworth, A. Nicholson, M. R. Appleby, and K. E. Orr. J. Clin. Pathol. 2003 Jul; 56(7): 528–531.
2. D. Fallon, N. Andrews, D. Frodsham, B. Gee, S. Howe, A. Iliffe, K. J. Nye, and R. E. Warren J. Clin. Pathol. 2002 Jul; 55(7): 524–529.

Ordering information

Product	Type	Cat. N°	Pack size
Chrom*Art* CHROMOGENIC URINE AGAR IV	DCM	409810G2	500 g (10,1 L)
Chrom*Art* CHROMOGENIC URINE AGAR IV	Ready to use plates (Ø90mm)	549810G	20 plates

IVD CE

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