BD™ CHROMagar™ Orientation Medium / Columbia CNA Agar (Biplate)

INTENDED USE

BD CHROMagar Orientation Medium / Columbia CNA Agar (Biplate) is used for isolation of bacteria commonly involved in urinary tract infections. While **CHROMagar** Orientation Medium is a non-selective medium for the isolation, identification, or differentiation of urinary tract pathogens, Columbia CNA Agar is a selective medium for the isolation of Gram positive bacteria.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

CHROMagar Orientation Medium: *Escherichia coli,* enterococci, the *Klebsiella-Enterobacter-Serratia* and the *Proteus-Morganella-Providencia* groups are the most frequent organisms producing urinary tract infections (=UTI). Sixty to 70% of UTI are caused by *E. coli* in pure culture or together with enterococci. *Staphylococcus saprophyticus* and *Streptococcus agalactiae* are, although less frequently, encountered in UTI in females.

Some of the organisms involved produce enzymes either for the metabolism of lactose or glucosides or both, whereas others produce none of these enzymes. As an example, *E. coli* produces enzymes of the lactose metabolism but is ß-glucosidase negative. Other members of the family *Enterobacteriaceae* are ß-glucosidase positive but do not contain enzymes necessary for lactose fermentation, and others may contain both types of enzymes or none of them. Beta-glucosidases are also found in Gram positive cocci such as *Enterococcus* spp. and *Streptococcus agalactiae*. Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group of organisms.

CHROMagar Orientation Medium allows the identification of *E. coli*, enterococci, and most *Staphylococcus saprophyticus* and *S. simulans* strains directly on the isolation plate; furthermore, the detection of the *Klebsiella-Enterobacter-Serratia* (=KES) and *Proteus-Morganella-Providencia* (=PMP) groups is possible by means of the colony and medium coloration.[1-3] As **CHROMagar** Orientation Medium is non-selective, other UTI pathogens will grow, but biochemical tests are needed for their identification.

CHROMagar Orientation Medium was developed by A. Rambach and is sold by BD Diagnostic Systems under a licensing agreement with CHROMagar, Paris, France.

In **CHROMagar** Orientation Medium specially selected peptones supply the nutrients. The chromogen mix consists of artificial substrates (chromogens) which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the direct differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests.

Columbia CNA Agar: Ellner et al. reported the development of a blood agar formulation, which has been designated as Columbia Agar.[4] This medium which achieves larger colonies and more luxuriant growth than on comparable blood agar bases, is utilized for media containing blood and for selective formulations. Ellner et al. found that a medium containing 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia agar base, enriched with 5% sheep blood, supports the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus, Klebsiella* and *Pseudomonas* species.[4]

Columbia Agar provides a highly nutritious base medium. The addition of the antimicrobial agents, colistin and nalidixic acid renders the medium selective for gram-positive micro-organisms, especially streptococci and staphylococci. Sheep blood is added to the medium to allow detection of hemolytic reactions.[4,5]

REAGENTS

BD CHROMagar Orientation Medium / Columbia CNA Agar (Biplate)

Approximate Formulas* Per Liter Purified Water

CHROMagar Orientation Medium	
Chromopeptones	16.1 g
Chromogenic Mix	1.3
Agar	15.0

pH 6.9 ± 0.2

Columbia CNA Agar			
Pancreatic Digest of Casein	12.0 g	Sodium Chloride	5.0 g
Peptic Digest of Animal Tissue	5.0	Agar	13.5
Yeast Extract	3.0	Colistin	0.01
Beef Extract	3.0	Nalidixic Acid	0.015
Corn Starch	1.0	Sheep Blood, defibrinated	5%

pH 7.3 ± 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates **in the dark** at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C. **Minimize exposure to light before and during incubation, since it will destroy the chromogens included in CHROMagar Orientation Medium**.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates aerobically at 35 to 37° C for not less than 20 to 24 hours.

Test strains	CHROMagar Orientation Medium	Columbia CNA Agar
Enterococcus faecalis ATCC™ 29212	Growth good to excellent; colonies small, blue-green to blue	Growth good to excellent; grey colonies; no hemolysis
Streptococcus agalactiae ATCC 12386	Growth fair to good; colonies tiny, blue	Growth fair to excellent; white to gray colonies; beta-hemolysis
Staphylococcus aureus ATCC 25923	Growth good to excellent; colonies white to yellowish	Growth good to excellent; whitish colonies, beta-hemolysis
Escherichia coli ATCC 25922	Growth fair to excellent; colonies medium-sized, transparent, pink	Inhibition complete
Klebsiella pneumoniae ATCC 27736	Growth good to excellent; colonies medium-sized, deep blue, with or without blue halo	Inhibition partial to complete

Proteus vulgaris ATCC 8427	Growth fair to excellent; blue-green colonies; surrounding medium is amber to brown	Inhibition partial to complete
Uninoculated	Colorless to very light amber, transparent (may contain up to a moderate amount of small particles)	Red (blood color)

PROCEDURE

Materials Provided

BD CHROMagar Orientation Medium / Columbia CNA Agar (90 mm **Stacker™** biplates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD CHROMagar Orientation Medium / Columbia CNA Agar is exclusively used for isolating, enumerating and differentiating bacteria in urine. Midstream or catheter urine, or urine collected by suprapubic bladder puncture can be used (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe aseptic techniques for collecting urine specimens. Urine must be directly streaked on the medium not later than 2 hours after collection or must be kept refrigerated (not longer than 24 hours) to avoid overgrowth of the infectious agents or contaminants before inoculation of this medium.[6-8]

Test Procedure

Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml). For the inoculation of media in biplates, 0.001 ml (= 1 µl) loops are preferred. Ensure proper loading of the loop with the specimen. First, inoculate the sample onto a small area of the surface of the **CHROMagar** Orientation Medium; then inoculate the remaining area of this medium. Afterwards, collect a new sample from the urine and proceed in the same way for Columbia CNA Agar. If 10 µl loops are used, a tenfold predilution of the urine specimen in sterile physiological saline is recommended. Incubate the inoculated plate in an inverted position at 35 to 37° C aerobically for 20 to 24 hours. It is not recommended to incubate this biplate in an atmosphere enriched with carbon dioxide. **Minimize exposure to light during incubation as this might destroy the chromogens included in CHROMagar Orientation Medium.** Once the colors of the colonies have developed, exposure to light is permissible.
Use of calibrated loops or other techniques commonly used for the plating of urine specimens is mandatory to obtain isolated colonies with their typical colors and shapes.

Results

After incubation, the media should show isolated colonies in the areas where the inoculum was appropriately diluted. **Scheme 1** should be used for the identification or differentiation and as a guideline for additional confirmatory reactions on **CHROMagar** Orientation Medium. A Gram stain and microscopy can be used to confirm results.
On Columbia CNA Agar, growth will occur if Gram positive bacteria are present. For details and interpretation of growth on this medium, consult the references.[5,9]

Confirmatory Tests

For **CHROMagar** Orientation Medium, perform the confirmatory tests as required (**Scheme 1**). Do not apply any detection reagent, such as DMACA indole or other reagents directly onto the colonies on this medium. Instead, the tests should be performed on filter paper with growth from respective colonies.
Do not use Kovac's indole reagent for E. coli colonies, as their color might interfere with the red color of a positive indole test; instead, use dimethylaminocinnamaldehyde (DMACA) indole reagent.

If other confirmatory tests or biochemical identification systems are used, the instructions of these tests or systems must be followed.

Calculation and Interpretation of Results [6-9]
Count the number of colonies (cfu) on each of the media. If a 0.001 ml loop was used, each colony corresponds to 1000 CFU/ml of urine.[7]
Midstream and catheter urine: Current guidelines indicate that for a single isolate a density of $\geq 10^5$ cfu/ml indicates infection, $<10^5$ cfu/ml indicates urethral or vaginal contamination, and between 10^4 to 10^5 CFU/ml needs to be re-evaluated based on clinical information. Contaminant bacteria usually appear in low numbers which vary in colonial morphology.
Urine collected by suprapubic bladder puncture: Since the bladder is sterile in non-infected individuals, any cfu detected indicates an infection.

Urinary tract pathogens will usually yield high counts having uniform colony morphology and color on this medium.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD CHROMagar Orientation Medium / Columbia CNA Agar (Biplate) is used for the isolation, identification or presumptive identification of bacteria commonly involved in urinary tract infections.
CHROMagar Orientation Medium is a chromogenic medium for the direct identification, differentiation and enumeration of common urinary tract pathogens. The medium is suitable for the isolation of many aerobically growing micro-organisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, enterococci, staphylococci, and many others from urine specimens. [1-3]
It allows the direct identification of *Escherichia coli* by colony color and a confirmatory indole test, and the direct identification of *Enterococcus* and *Streptococcus agalactiae* by colony color and a confirmatory PYR test, or alternatively, a serological agglutination test for the determination of the Lancefield group. Other organisms can either be identified after performing a few confirmatory tests or may require a full biochemical identification, depending on the species. Since most of the common urinary tract infections are caused by *E. coli* and/or enterococci, the use of this medium significantly reduces the workload and time for inoculating and reading identification systems which are necessary when conventional media are used for the isolation.
Columbia CNA Agar is a standard medium for the isolation and cultivation of many aerobically growing Gram positive micro-organisms, e.g., streptococci, staphylococci, coryneforms, *Listeria* spp and others.[5,7,9]

Performance Results [2,10]
Two independent performance evaluations were conducted on **CHROMagar** Orientation Medium. In both evaluations, the chromogenic medium detected more pathogens than the traditional media used for comparison. Details on the first evaluation have been published, and results of the first and the second evaluation are available from the **Instructions for Use** document of **BD CHROMagar Orientation Medium** (cat. no. 254102).[2]

Limitations of the Procedure
CHROMagar Orientation Medium: Colonies that show their natural color and do not react with the chromogenic substrates must be further differentiated with appropriate biochemical or serological tests. Consult the references.[9,11]
Gram negative rods other than those belonging to the KES group may produce large blue colonies and thus require additional biochemical tests for their identification.[11]
In very rare cases, *Listeria monocytogenes* or other *Listeria* species might be present in urine (e.g. after abortion due to these agents). *Listeria* will produce small blue to blue-green colonies that are PYR-negative, mimicking *Streptococcus agalactiae* (see Scheme 1). Therefore, it may be useful to prepare a Gram stain of all strains producing small, blue to blue-green colonies on this medium that are PYR negative. The presence of Gram positive rods may be indicative for *Listeria* species. Additional biochemical tests are necessary to confirm the presence of these agents.

Very rarely, isolates of *Aeromonas hydrophila* may produce rose to pink colonies. They may be differentiated from *E. coli* with an oxidase test (*Aeromonas* = positive; *E. coli* = negative). Occasionally, coagulase-negative staphylococci other than *S. saprophyticus*, e.g. *S. simulans, S. xylosus,* and *S. intermedius,* may take on a rose to pink colony color. Therefore it is necessary to perform additional tests (see Scheme 1) on these isolates.
CHROMagar Orientation Medium will not support the growth of fastidious organisms such as *Neisseria*, *Haemophilus*, or *Mycoplasma* spp.
Use of this medium for non-clinical or clinical specimens other than urine has not been validated.
Before using **CHROMagar** Orientation Medium for the first time, it is recommended to train the typical colony appearance with defined strains, e.g., the strains mentioned under **USER QUALITY CONTROL**.

Columbia CNA Agar: Bacteria exhibiting resistance to the selective ingredients may grow on this medium.
Candida species and other fungi are not inhibited on this medium.
Although they are Gram positive bacteria, aerobic spore-formers such as *Bacillus* spp., may be inhibited on Columbia CNA Agar with 5% Sheep Blood.
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.
Columbia Agar base has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

REFERENCES

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PACKAGING/AVAILABILITY

BD CHROMagar Orientation Medium / Columbia CNA Agar (Biplate)

REF 254489	Ready-to-use Plated Media, cpu 20
REF 257727	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.

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Scheme 1: Guidelines for the appearance of colonies, for the performance of confirmatory tests and the resulting differentiation or identification on BD CHROMagar Orientation Medium

Small, blue-green to blue colonies:
↓
PYR[b] test
↓ ↓
red (+) colorless (-)[a]
Enterococcus* spp.** **Cocci: *Str. agalactiae
Rods: *Listeria* species

:

Large, blue colonies:
***Klebsiella-Enterobacter-Serratia* group (KES)**
↓
Identify species with biochemical test system

Large pink, transparent colonies:
↓
DMACA[c] indole test
↓ ↓
green (+) colorless to red (-)
E. coli [d] Identify species with biochemical test system

Small opaque, pink to rose colonies:
↓
Novobiocin 5 µg disc[e]
↓ ↓
resistant susceptible
Staphylococcus saprophyticus[f]*, S. xylosus*[f] ***S. simulans*** [f] *S. intermedius* [g]
↓ ↓
Identify species with biochemical test system

Colorless to beige colonies on an amber to brown medium [h]**:**
***Proteus-Morganella-Providencia* group PMP**
↓
DMACA [c] indole test
↓ ↓

green (+)	colorless to rose (-)
Proteus vulgaris H_2S + [i]	*Proteus mirabilis* (ODC +) [j]
Providencia spp. H_2S -	*Proteus penneri* (ODC -)
Morganella spp. H_2S -	

[a] Gram stain recommended.
[b] Pyroglutamate test for pyrrolidonyl arylamidase. Serological tests for the Lancefield grouping may be used instead of a PYR test for differentiation of *Enterococcus* spp. from *Streptococcus agalactiae.*
[c] DMACA= Dimethylamino cinnamaldehyde reagent for indole production. Apply reagent on filter paper and rub one colony into the area containing reagent on the filter paper. Wait for 10-20 sec. A **green** color is indicative of indole production (red or colorless = negative). Do not use Kovac's indole reagent for testing pink colonies!
[d] An oxidase test may be performed on all indole positve, large pink colonies to exclude *Aeromonas* (=oxidase positive).
[e] Spread-inoculate a Mueller Hinton II Agar plate with the isolate. Place a novobiocin (5 µg disc) on the inoculated plate. Incubate for 18 to 24 hours at 35 to 37° C and measure the inhibition zone size (resistant: ≤ 16 mm, susceptible: > 16 mm).
[f] Known as human pathogens, may also be isolated from urine.
[g] Not known to be isolated from human urine.
[h] The amber to brown color is due to positive tryptophan deaminase (TDA) common to all PMP group organisms. About 50% of *P. vulgaris* strains produce blue colonies on an amber to brown medium
[i] Conventional hydrogen sulfide test.
[j] Conventional ornithine decarboxylase test.