BD™ CHROMagar™ Orientation Medium

INTENDED USE

BD CHROMagar Orientation Medium is a nonselective medium for the isolation, direct identification, differentiation and enumeration of urinary tract pathogens. **BD CHROMagar Orientation Medium** allows for the differentiation and identification of *Escherichia coli* and *Enterococcus* without confirmatory testing.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.
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.
Due to the different antimicrobial susceptibilities of the agents involved, their species identification with batteries of biochemical tests is necessary for effective antimicrobial therapy. This is one of the most time-consuming tasks in a laboratory processing urine specimens. The most frequently isolated species or organism groups produce characteristic enzymes. Thus, it is possible to identify these organisms to the species level with a limited number of substrate fermentation or utilization tests.[1,2]

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.

Performance evaluations have demonstrated that **BD CHROMagar Orientation Medium** is superior to commonly used differential media for the isolation, differentiation and counting of UTI pathogens, such as CLED agar or a combination of Blood and MacConkey Agars.[3-5] **BD CHROMagar Orientation Medium** allows for the identification of *E. coli* and enterococci directly on the isolation plate; furthermore, the presumptive identification of most *Staphylococcus saprophyticus* and *S. agalactiae* strains, as well as the *Klebsiella-Enterobacter-Serratia* (=KES) and *Proteus-Morganella-Providencia* (=PMP) groups is possible by means of the colony and medium coloration. As **BD 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 under a licensing agreement with CHROMagar, Paris, France.

In **BD 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.

REAGENTS

BD CHROMagar Orientation Medium

Formula* Per Liter Purified Water

Chromopeptone	16.1 g
Chromogen Mix	1.3
Agar	15.0

pH 6.9 +/- 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.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the plates in an inverted position at 35 to 37° C aerobically for 20 to 24 hours.

Strains	Growth Results
Escherichia coli ATCC™ 25922	Growth good to excellent; colonies medium-sized to large, dark rose to pink, transparent
Enterobacter cloacae ATCC 13047	Growth good to excellent; colonies medium-sized, deep blue, with or without violet halos
Proteus mirabilis ATCC 14153	Growth good to excellent; colonies medium-sized, pale to beige, surrounded by an amber to brown halo; in areas of dense growth, the medium may be completely amber to brown. Swarming is partially to completely inhibited.
Enterococcus faecalis ATCC 29212	Growth good to excellent; colonies small, blue-green to blue
Streptococcus agalactiae ATCC 12386	Growth fair to good; colonies pinpoint to small, light blue-green to light blue, with or without halos
Staphylococcus aureus ATCC 25923	Growth good to excellent; colonies medium sized to small, with their natural color (white to cream)
Staphylococcus saprophyticus ATCC 15305 (=NCTC 10516)	Growth fair to good; colonies small, opaque, light pink to rose
Uninoculated	Colorless to very light amber, transparent

PROCEDURE

Materials Provided

BD CHROMagar Orientation Medium (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used exclusively for 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.

Test Procedure

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.
Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml). Ensure proper loading of the loop with the specimen. Inoculate the sample down the middle of the plate in a single streak from which additional spreading of the inoculum is performed. [6,7]
Incubate the inoculated plates in an inverted position at 35 to 37° C aerobically for 20 to 24 hours. **Avoid exposure to light during incubation as this might destroy the chromogens.** Once the colors of the colonies have developed, exposure to light is permissible.

Results

After incubation, the plates should show isolated colonies in the areas where the inoculum was diluted appropriately. Table 1 and Scheme 1 should be used for identification or differentiation and as a guideline for additional confirmatory tests. A Gram stain and microscopy can be used to confirm results.

Table 1 Guidelines for Identification Based on Different Colony Colors

Organism	**Appearance on BD CHROMagar Orientation Medium**	**Confirmatory Tests** (Necessary for further differentiation)
E. coli [a]	Medium-sized to large, dark rose to pink, transparent colonies with or without halos in the surrounding medium	
KES [b] group	Medium-sized, blue to dark blue colonies, with or without violet halos	**BBL™ CRYSTAL™ E/NF** for differentiation within the genera
PMP [c] group	Pale to beige colonies surrounded by brown halos [d]	Indole, H_2S [e], ODC[f], **BBL CRYSTAL E/NF** for differentiation within the genera
Enterococcus	Small blue-green colonies	
S. agalactiae	Light blue-green to blue, pinpoint to small colonies with or without halos	PYR [g]
S. saprophyticus (most strains)	Light pink to rose, small opaque colonies with or without halos	5 µg novobiocin disc [h]
Other (including yeasts)	Natural (cream) pigmentation	Appropriate biochemical or serological identification tests

For footnotes a-h, see Scheme 1

Confirmatory Tests

Perform the confirmatory tests as required (Table 1, Scheme 1). Do not apply any detection reagent, directly onto the colonies on **BD CHROMagar Orientation Medium**. Instead, the tests should be performed on filter paper with growth from respective colonies.
For E. coli colonies that are dark rose to pink, but are pinpoint to small in size, do not use Kovacs' indole reagent, as their colony color may interfere with the red color of a positive indole test; instead, use dimethylaminocinnamaldehyde (DMACA) indole reagent (green = positive).
If other confirmatory tests or biochemical identification systems are used, the instructions accompanying these tests or systems must be followed.
Perform confirmatory testing for *Enterococcus* only if speciation beyond the genus level is required.

Scheme 1: Guidelines for the Performance of Identification Tests on Selected Organisms

Colony Appearance				
Small, rose, opaque	⇒ 5 µg novobiocin disc	⇒ sensitive	⇒*S. intermedius, S. simulans*	**BBL CRYSTAL GP** for differentiation within the genera
		⇒ resistant	⇒ *S. sapro-phyticus, S. xylosus*	
Colorless to beige colonies, orange-brown medium	⇒ PMP group	⇒ DMACA [i] Indole test	⇒ green (positive) ⇓	
			H_2S positive [e]	⇒ *P. vulgaris*
			H_2S negative [e]	⇒*Providencia* spp., *Morganella* spp.
			⇒ colorless to rose (negative	
			ODC positive [f]	⇒ *P. mirabilis*
			ODC negative [f]	⇒ *P. penneri*

[a] See **Limitations of the Procedure**
[b] KES = *Klebsiella-Enterobacter-Serratia* group
[c] PMP = *Proteus-Morganella-Providencia* group
[d] 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.
[e] Conventional hydrogen sulfide test.
[f] Conventional ornithine decarboxylase test.
[g] Pyroglutamate test for pyrrolidonyl arylamidase.
[h] 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 determine the inhibition zone size. (resistant: ≤ 16 mm, susceptible: > 16 mm).
[i] DMACA= Dimethylaminocinnamaldehyde 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).

Calculation and Interpretation of Results [6,7]

Count the number of colonies (cfu) on the plate. If a 0.01 ml loop was used, each resultant colony is representative of 100 CFU/ml; 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.[7] 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 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. Performance evaluations have demonstrated that **BD CHROMagar Orientation Medium** has advantages over other differential media used in the isolation, differentiation and enumeration of UTI pathogens, such as CLED Agar or a combination of Blood and MacConkey Agars.[3-5]

BD CHROMagar Orientation Medium allows for the differentiation and identification of *E. coli* and enterococci without confirmatory testing, based on the criteria for identification established by the CLSI standard M35-A, "Abbreviated Identification of Bacteria and Yeast; Approved Guideline." [8] Presumptive identification of *S. saprophyticus*, *S. agalactiae, Klebsiella-*

Enterobacter-Serratia (KES) and the *Proteus-Morganella-Providencia* (PMP) groups is possible by means of colony morphology, pigmentation and medium discoloration.

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.

Performance Results [4,9,10]

The microbiological performance of **BD CHROMagar Orientation Medium** and a chromogenic competitor medium were compared to that of Columbia agar with 5% sheep blood and MacConkey agar without crystal violet for the enumeration and presumptive identification of bacteria responsible for urinary tract infections.[4] Of a total of 658 clinical urine specimens, 118 specimens yielded no growth, 402 specimens yielded growth with cell counts of $\geq 10^5$ CFU/ml, and 138 specimens yielded growth with cell counts of $<10^5$ CFU/ml. Of the specimens with cell counts of $\geq 10^5$ CFU/ml, 163 were pure cultures and 239 were mixed cultures. A total of 266 *Escherichia coli* isolates were obtained on both chromogenic media, 260 were isolated on blood agar, and 248 were isolated on MacConkey agar. One strain (0.4%) failed to develop the expected pink color on **BD CHROMagar Orientation Medium**, and 23 strains (8.7%) failed to develop the expected pink color on the competitor medium. Enterococci (**BD CHROMagar Orientation Medium**, *n* = 266; competitor medium, *n* = 265) produced small blue-green colonies on both chromogenic media. Fifty of the mixed cultures contained enterococci that were detected only on the chromogenic media. The *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups could be identified on both chromogenic media. Of 66 isolates of the KES group, 63 grew with the expected color on **BD CHROMagar Orientation Medium** and 58 of 64 isolates grew with the expected color on the competitor medium. Other microorganisms required further identification tests.

In a second performance evaluation with a total of 421 clinical urine specimens, 286 of which yielded growth of bacteria or yeasts, **BD CHROMagar Orientation Medium** was compared to **BD Columbia Agar with 5% Sheep Blood**.[9] On the chromogenic medium, 483 isolates were obtained, and 447 strains were isolated on the blood agar medium. Strains were identified biochemically. Of the *E. coli* strains, 95% were correctly identified by their rose to pink colony color, while the remaining *E. coli* strains were nonpigmented. All isolates of *Enterococcus* spp. showed the typical blue to blue-green colony color. *Streptococcus agalactiae* isolates produced tiny, light blue to blue-green colonies and were differentiated from enterococci by a negative PYR test. All *Staphylococcus saprophyticus* and *S. simulans* isolates produced small rose opaque colonies and were differentiated with a novobiocin test and identified to the species level with biochemical tests.

In a blinded internal study which included testing of over 900 bacterial strains seeded in urine, the sensitivity and specificity of **BD CHROMagar Orientation Medium** identification of *E. coli*, based on colony color and morphology only, were 97% and 99%, respectively; for *Enterococcus* the sensitivity and specificity of identification were 99% and 97%, respectively (see table).[10]

Organism	**Sensitivity % (95% Confidence Interval)**	**Specificity % (95% Confidence Interval)**
E. coli	277/286 96.9% (94.1-98.6%)	638/645 98.9% (97.8-99.6%)
Enterococcus	319/324 98.5% (96.4-99.5%)	603/622 97% (95.3-98.2%)

Limitations of the Procedure

As this medium is nonselective, other UTI pathogens will grow. Colonies that show their natural color and do not react with the chromogenic substrates on **BD CHROMagar Orientation Medium** must be further differentiated with appropriate biochemical or serological tests. Consult the references.[1,2]

E. coli colonies that are dark rose to pink but are pinpoint to small in size, require additional confirmatory tests such as spot indole (DMACA indole reagent).
Gram negative rods other than those belonging to the KES group may produce large blue colonies on **BD CHROMagar Orientation Medium** 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*. Therefore, it may be useful to prepare a Gram stain of all strains producing tiny to 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, but additional biochemical tests are necessary to confirm their identification.

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,* produce small rose opaque colonies. Therefore, it is necessary to perform additional tests (see Scheme 1) on these isolates.

BD 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 documented.

Before using **BD CHROMagar Orientation Medium** for the first time, we recommend to train the typical colony appearance with defined strains, e.g., the strains mentioned under **USER QUALITY CONTROL**.

REFERENCES

1. Isenberg, H.D. (ed.). 1992. Clinical Microbiology Procedures Handbook, vol. 1. American Society for Microbiology. Washington, DC.
2. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis.
3. Merlino, J., S. Siarakas, G. J. Robertson, G. R. Funnell, T. Gottlieb, and R. Bradbury. 1996. Evaluation of CHROMagar Orientation for differentiation and presumptive identification of gram-negative bacilli and *Enterococcus* species. J. Clin. Microbiol. *34*: 1788-1793.
4. Hengstler, K.A., R. Hammann, and A.-M. Fahr. 1997. Evaluation of BBL CHROMagar Orientation medium for detection and presumptive identification of urinary tract pathogens. J. Clin. Microbiol. *35*: 2773-2777.
5. Samra, Z., M. Heifetz, J. Talmor, E. Bain, and J. Bahar. 1998. Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. J. Clin. Microbiol. *36*: 990-994.
6. Clarridge, J.E., M.T. Pezzlo, and K.L. Vosti. 1987. Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
7. Forbes, B.A., and P.A. Granato. Processing specimens for bacteria. 1995. *In:* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Approved Guideline M35. Abbreviated identification of bacteria and yeast, CLSI, Wayne, PA.*Search for latest version at* **www.clsi.org**
9. Data on file. BD Diagnostic Systems Europe, Heidelberg, Germany.
10. Data on file. BD Diagnostic Systems, Sparks, MD, USA.
11. Abbott, S.L. 2003. *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas,* and other *Enterobacteriaceae. In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD CHROMagar Orientation Medium

Cat. No. 257481	Ready-to-use Plated Media, cpu 20
Cat. No. 254107	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/

CHROMagar is a trademark of Dr. A. Rambach
ATCC is a trademark of the American Type Culture Collection
BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD