

# **BBL™ CHROMagar™ Orientation**

## **I. INTENDED USE**

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

## **II. SUMMARY AND EXPLANATION**

*Escherichia coli*, enterococci, the *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups are frequently encountered organisms in urinary tract infections (UTI). Most UTIs are caused by *E. coli* alone, or in combination with enterococci. *Staphylococcus saprophyticus* and *Streptococcus agalactiae* may be isolated from females, although less frequently.

Due to the different antimicrobial susceptibility patterns of the microorganisms involved, identification to the species level is necessary for effective antimicrobial therapy. 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.<sup>1</sup>

Some of the organisms encountered in UTIs produce enzymes either for the metabolism of lactose or glucosides or both. Other organisms produce none of these enzymes. For example, *E. coli* contains enzymes for lactose metabolism but is  $\beta$ -glucosidase negative. Some members of the family *Enterobacteriaceae* are  $\beta$ -glucosidase positive but do not contain enzymes necessary for lactose fermentation; others may contain both types of enzymes or none of them.  $\beta$ -glucosidases are also found in gram-positive cocci, such as *S. agalactiae* and the enterococci. Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group.

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

## **III. PRINCIPLES OF THE PROCEDURE**

Specially selected peptones supply the nutrients in **BBL CHROMagar** Orientation medium. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests. *Proteus* swarming is partially to completely inhibited.

#### IV. REAGENTS

##### **BBL CHROMagar Orientation**

Approximate Formula\* Per Liter Purified Water

Chromopeptone.....	16.1 g
Chromogen Mix.....	1.3 g
Agar .....	15.0 g

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

##### ***Warnings and Precautions:***

For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over the off-set lid and allow to air dry in order to prevent formation of a seal between the top and the bottom of the plate during incubation.

***Storage Instructions:*** On receipt, store plates in the dark at 2 – 8°C in original sleeve wrapping and box until time of inoculation. Plates may be used up until the expiration date.

***Product Deterioration:*** Do not use plates if they show evidence of microbial contamination, discoloration, drying or cracking.

#### V. SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>5-8</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

#### VI. PROCEDURE

***Material Provided:*** BBL CHROMagar Orientation

***Materials Required But Not Provided:*** Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required for this procedure.


***Test Procedure:*** Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Allow the medium to warm to room temperature before inoculation.

A dilution of the specimen on the plate (by using calibrated loops or other techniques commonly used for plating urine specimens) is required to obtain isolated colonies with typical colors and morphology. Incubate plates aerobically at 35 ± 2°C for not less than 20 to 24 h in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide.

Avoid exposure to light during incubation as light may destroy the chromogens. Once the colony color develops, exposure to light is permissible.

## VII. USER QUALITY CONTROL

Examine plates for signs of deterioration as described under "Product Deterioration." Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

<i>Test Strain</i>	<i>Expected Results</i>
 <i>Enterobacter cloacae</i> ATCC™ 13047	Growth; medium size, dark blue to medium-blue colonies with or without violet halos in the surrounding medium
<i>Enterococcus faecalis</i> ATCC 29212	Growth; small size, blue-green colonies
<i>Escherichia coli</i> ATCC 25922	Growth; medium to large size, transparent, dark rose to pink colonies with or without halos
<i>Proteus mirabilis</i> ATCC 43071	Growth; medium size, transparent, pale beige to brown colonies, surrounded by a brown halo. In areas of dense growth, the medium may be completely orange-brown. Swarming is partially to completely inhibited.
<i>Staphylococcus aureus</i> ATCC 25923	Growth; small to medium size, white to cream colonies (natural pigmentation)
<i>Streptococcus agalactiae</i> ATCC 12386	Growth; pinpoint to small size, light blue-green to light blue colonies with or without halos.

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

## VIII. 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 reactions. A Gram stain and microscopic examination can be used to confirm results.

**Confirmatory Tests:** **BBL CHROMagar Orientation** has been validated as an acceptable medium for both identification and antimicrobial susceptibility testing on the **BD Phoenix™** System.

Do not apply any detection reagents directly onto the colonies growing on the medium. Perform the tests on filter paper with growth from the 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 the colony color may interfere with the red color of a positive indole test. Use only dimethylaminocinnamaldehyde (DMACA) indole reagent.

If other confirmatory tests or biochemical identification systems are used, follow the instructions accompanying the identification systems.

Perform confirmatory testing for *Enterococcus* only if speciation beyond the genus level is required.

**Table 1: Guidelines for Identification Based on Different Colony Colors**

<i>Organism</i>	<i>Appearance on BBL CHROMagar Orientation Medium</i>	<i>Confirmatory Tests (Necessary for further differentiation)</i>
<i>E. coli</i> *	Dark rose to pink, transparent colonies, medium to large size, with or without halos in the surrounding medium	
KES group	Medium-blue to dark blue colonies	<b>BBL™ Crystal™</b> E/NF for differentiation within the genera
PMP group	Pale to beige colonies surrounded by brown halos**	Indole, H <sub>2</sub> S, ODC, <b>BBL Crystal</b> E/NF for differentiation within the genera
<i>Enterococcus</i>	Blue-green small colonies	
<i>S. agalactiae</i> *	Light blue-green to light blue, pinpoint to small colonies, with or without halos	PYR
<i>S. saprophyticus</i> (most strains)	Light pink to rose, small opaque colonies with or without halos	5 µg Novobiocin disc
Other including yeasts	Natural (cream) pigmentation	Appropriate biochemical or serological identification methods

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

<b>Colony Appearance</b>				
Small, rose, opaque	× Novobiocin 5 µg disc	× Sensitive	× <i>S. intermedius</i>	
		× Resistant	× <i>S. xylosum</i>	
			× <i>S. saprophyticus</i>	× Identify species with biochemical tests
Colorless to beige colonies, orange-brown medium	× PMP group	× DMACA	× green (positive)	
			↓	
			H <sub>2</sub> S positive	× <i>P. vulgaris</i>
			H <sub>2</sub> S negative	× <i>Providencia</i> spp.
			H <sub>2</sub> S negative	× <i>Morganella</i> spp.
			× colorless to rose (negative)	
			↓	
ODC positive	× <i>P. mirabilis</i>			
ODC negative	× <i>P. penneri</i>			

\* See "Limitations of the Procedure."

\*\* About 50% of *P. vulgaris* strains produce blue colonies on a brownish halo.

Key: KES = *Klebsiella-Enterobacter-Serratia* group  
PMP = *Proteus-Morganella-Providencia* group  
ODC = Conventional ornithine decarboxylase test  
H<sub>2</sub>S = Conventional hydrogen sulfide test  
DMACA = Indole test performed with DMACA (dimethylaminocinnamaldehyde) reagent.

**IX. 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 must be further differentiated with appropriate biochemical or serological tests to confirm identification.

*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 organisms other than those belonging to the KES group may produce large blue colonies and thus require other biochemical tests for identification.

In very rare cases, *Listeria monocytogenes* or other *Listeria* species may be present in urine (e.g., after abortion due to these agents). *Listeria* will produce blue to blue-green colonies that are PYR negative, mimicking *Streptococcus agalactiae*. Therefore, it may be useful to perform a Gram stain of organisms producing small, blue to blue-green colonies on this medium that are PYR negative. The presence of gram-positive bacilli may be indicative of *Listeria* species, but additional biochemical tests are necessary to confirm their identification.

Very rarely, isolates of *Aeromonas hydrophila* may produce rose colonies. They may be differentiated from *E. coli* with the oxidase test (*Aeromonas* = positive; *E. coli* = negative).

This medium will not support the growth of fastidious organisms, such as *Neisseria* spp., *Haemophilus* spp. or *Mycoplasma* spp.

Use of this medium for non-clinical or clinical specimens other than urine has not been documented.

Minimize exposure of **BBL CHROMagar** Orientation medium to light before and during incubation, as light may destroy the chromogens.

Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

## X. PERFORMANCE CHARACTERISTICS

Clinical studies have demonstrated that **BBL 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.<sup>2-4</sup>

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.

Further testing must be performed for confirmation. (See Table 1)

**BBL 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 NCCLS standard M35-A, "Abbreviated Identification of Bacteria and Yeast; Approved Guideline."<sup>9</sup> In a blinded internal study which included testing of over 900 bacterial strains seeded in urine, the sensitivity and specificity of **BBL CHROMagar** Orientation 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).

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

## XI. AVAILABILITY

<i>Cat. No.</i>	<i>Description</i>
254102	<b>BBL™ CHROMagar™</b> Orientation, Pkg. of 20
215081	<b>BBL™ CHROMagar™</b> Orientation, Ctn. of 100

## XII. REFERENCES

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