BD BBL™ Trypticase™ Soy Agar with 5% Sheep Blood // BD BBL™ CHROMagar™ Orientation

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

BD BBL™ Trypticase™ Soy Agar with 5% Sheep Blood is used for the growth of fastidious organisms and for the visualization of hemolytic reactions.

BD BBL™ CHROMagar™ Orientation is a nonselective medium for the isolation, differentiation and enumeration of urinary tract pathogens.

II PERFORMANCE TEST PROCEDURE

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Using a volumetric pipettor or equivalent method, deliver 0.1 mL of a dilution yielding 30 – 300 CFU to each plate and spread-inoculate using a sterile glass spreader.
   b. Incubate the *Staphylococcus* and *Escherichia* strains at 35 ± 2 °C in an aerobic atmosphere and the *Streptococcus* strains at 35 ± 2 °C in an aerobic atmosphere supplemented with 3 – 5% carbon dioxide.
2. Examine plates after 18–24 h for growth, colony size and hemolytic reactions.
3. Expected Results

CLSI Organisms	ATCC	Recovery
**Streptococcus pyogenes*	19615	Growth, beta hemolysis
**Streptococcus pneumoniae*	6305	Growth, alpha hemolysis
**Staphylococcus aureus*	13150	Growth, beta hemolysis
**Staphylococcus aureus*	25923	Growth
**Escherichia coli*	25922	Growth

*Recommended organism strain for User Quality Control.

B. BD BBL CHROMagar Orientation

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak inoculate with 10^3-10^5 CFUs of all organisms.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **BD BBL Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth and color formation.
3. Expected Results

Organisms	ATCC®	Recovery	Colony Color
**Enterobacter cloacae*	13047	Fair to heavy growth.	Dark blue to medium blue with or without violet halos in the surrounding medium
**Enterococcus faecalis*	29212	Fair to heavy growth of small size colonies.	Blue-green
**Escherichia coli*	25922	Fair to heavy growth of medium to large size colonies.	Transparent, dark rose to pink, with or without halos
**Proteus mirabilis*	12453	Fair to heavy growth of medium size colonies. Swarming is partially to completely inhibited.	Transparent, pale beige to brown, surrounded by a brown halo. In areas of dense growth, the medium may be completely orange-brown.
**Staphylococcus aureus*	25923	Fair to heavy growth of small to medium size colonies.	White to cream (natural pigmentation)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.9 ± 0.2 (**BD BBL CHROMagar** Orientation) and 7.3 ± 0.2 (**BD BBL Trypticase** Soy Agar with 5% Sheep Blood).
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1°C for 84 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BD BBL Trypticase™ Soy Agar with 5% Sheep Blood is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

U.S. Patent Nos. 5,716,799; 5,962,251

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

V SUMMARY AND EXPLANATION

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

The nutritional composition of **BD BBL Trypticase** Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. **BD BBL Trypticase** Soy Agar with 5% Sheep Blood is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially *Streptococcus* species.

B. BD BBL CHROMagar Orientation

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.[1]

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 b-glucosidase negative. Some members of the family *Enterobacteriaceae* are ß-glucosidase positive but do not contain enzymes necessary for lactose fermentation; others may contain both types of enzymes or none of them. ß-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.

VI PRINCIPLES OF THE PROCEDURE

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

The combination of casein and soy peptones in the **BD BBL Trypticase** Soy Agar base render the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.[2] Hemolytic reactions of streptococci are proper and growth of *Haemophilus haemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

BD BBL Trypticase Soy Agar with 5% Sheep Blood provides excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms. It is suitable for use with low concentration (0.04 unit) bacitracin discs (**BD Taxo™** A) for presumptive identification of group A streptococci (*S. pyogenes*).

B. BD BBL CHROMagar Orientation

Specially selected peptones supply the nutrients in **BD 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.

VII REAGENTS

BD BBL Trypticase Soy Agar with 5% Sheep Blood

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g	Agar	14.0 g
Papaic Digest of Soybean Meal	5.0 g	Growth Factors	1.5 g
Sodium Chloride	5.0 g	Defibrinated Sheep Blood	5%

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

BD 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 in Singapore.

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

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation time. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[3,4] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[5-8] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

IX PROCEDURE

Material Provided: BD BBL Trypticase Soy Agar with 5% Sheep Blood and **BD BBL CHROMagar** Orientation

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

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

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 20–24 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.

User Quality Control:

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 CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

Typical results on **BD BBL Trypticase** Soy Agar with 5% Sheep Blood are as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2–4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic
2. findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.)
3. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
4. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
5. *Listeria*. Small zones of beta hemolysis are produced. They may be distinguished by their rod shape in stains, and by motility at room temperature.
6. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

B. BD BBL CHROMagar Orientation

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: BD 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

Organism	Appearance on BD BBL CHROMagar Orientation Medium	Confirmatory Tests (Necessary for further differentiation)
*E. coli**	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.	**BD BBL™ Crystal™** E/NF for differentiation within the genera
PMP group	Pale to beige colonies surrounded by brown halos.**	Indole, H_2S, ODC, **BD BBL Crystal** E/NF for differentiation within the genera
Enterococcus	Blue-green small colonies.	
*S. agalactiae**	Light blue-green to light blue, pinpoint to small colonies, with or without halos.	PYR
S. saprophyticus (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

* 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_2S = Conventional hydrogen sulfide test; DMACA = Indole test performed with DMACA (dimethylaminocinnamaldehyde) reagent.

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

Colony Appearance

Small, rose, opaque	Novobiocin 5 µg disc	⇒ sensitive ⇒ resistant	⇒ *S. intermedius* ⇒ *S. xylosus* ⇒ *S. saprophyticus* ⇒ Identify species with biochemical tests
Colorless to beige colonies, orange-brown medium	⇒ PMP group	⇒ DMACA	⇒ green (positive) ⇓ H_2S positive ⇒ *P. vulgaris* H_2S negative ⇒ *Providencia* spp. H_2S negative ⇒ *Morganella* spp. ⇒ colorless to rose (negative) ⇓ ODC positive ⇒ *P. mirabilis* ODC negative ⇒ *P. penneri*

* 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_2S = Conventional hydrogen sulfide test; DMACA = Indole test performed with DMACA (dimethylaminocinnamaldehyde) reagent.

XI LIMITATIONS OF THE PROCEDURE

A. BD BBL CHROMagar Orientation

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 **BD 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.

B. Both Media

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for detailed information and recommended procedures.[3,4,9-11]

XII AVAILABILITY

Cat. No.	Description
252075	**BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood **// BD BBL™ CHROMagar™** Orientation Ctn. of 100 plates

XIII REFERENCES

1. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Vera, H.D., and D.A. Power. 1980. Culture media, p. 969. *In* E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53–80.
7. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021–0045.
9. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. J.B. Lippincott Company, Philadelphia.
10. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
11. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
12. 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.
13. 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.
14. 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.
15. Clinical and Laboratory Standards Institute. 2002. Approved Guideline M35-A. Abbreviated identification of bacteria and yeast, CLSI, Wayne, PA.
16. Fries, S.M. 1995. Diagnosis of group A streptococcal pharyngitis in a private clinic: comparative evaluation of an optical immunoassay method and culture. J. Pediatr. *126*: 933–936.
17. Nguyen, T.M., et al. 1998. Detection of group B streptococcus: comparison of an optical immunoassay with direct plating and broth-enhanced culture methods. J. Matern. Fetal. Med. 7:172–176.
18. Rossmann, S.N. et al. 1998. Isolation of *Lautropia mirabilis* from oral cavities of human immunodeficiency virus-infected children. J. Clin. Microbiol. *36*:1756–1760.
19. Isenberg, H.D., D. Goldberg, and J. Sampson, 1970. Laboratory studies with a selective medium. Appl. Microbiol. *20*: 433–436.

Technical Information: Please contact your local BD representative.

Nippon Becton Dickinson Co. Ltd.
1 Gotanda, Tsuchifune, Fukushima-shi, Fukushima-ken Japan
e-mail : BD-eDial@bd.com
WEB : www.bd.com/jp/