BD BBL™ CHROMagar™ Staph aureus / BBL™ CHROMagar™ MRSA II (Biplate)

INTENDED USE

BBL™ CHROMagar™ Staph aureus /BBL™ CHROMagar™ MRSA II (Biplate) is used for the isolation and identification of *Staphylococcus aureus* and for the qualitative direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens.

SUMMARY AND EXPLANATION

Staphylococcus aureus is a well documented pathogen. It is responsible for infections ranging from superficial to systemic.[1,2] Due to the prevalence of this organism and its clinical implications, detection is of utmost importance. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and life threatening infections. MRSA infections have been associated with a significantly higher morbidity, mortality and cost compared to methicillin-susceptible *S. aureus* (MSSA) infections. Selection of these organisms has been greatest in the healthcare setting; however, MRSA has also become more prevalent in the community.[3,4]

BBL CHROMagar Staph aureus is intended for the isolation, enumeration and identification of *S. aureus* based on the formation of mauve-colored colonies after 20 to 24 h incubation. The addition of chromogenic substrates to the medium facilitates the differentiation of *S. aureus* from other organisms.
BBL CHROMagar MRSA II (CMRSAII) is a selective and differential medium for the qualitative direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens. The test can be performed on respiratory, lower gastrointestinal (= GI), skin and wound specimens, on anterior nares specimens for the screening of nasal colonization to aid in the prevention and control of MRSA infections in healthcare settings and on positive blood culture bottles containing gram-positive cocci.

The combination of the two media in a biplate allows the isolation of *Staphylococcus aureus* and MRSA in one plate.

BBL CHROMagar Staph aureus and **BBL CHROMagar MRSA** were originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulations utilizing proprietary intellectual property used in the manufacturing of the prepared plated media.

PRINCIPLES OF THE PROCEDURE

Microbiological method.
In both media, specially selected peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and some grampositive cocci. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by specific enzymes. This facilitates the detection and differentiation of *S. aureus* from other organisms. *S. aureus* utilizes one of the chromogenic substrates, producing mauve-colored colonies. The growth of mauve-colored colonies at 24 h is considered positive for *S. aureus* and MRSA on **BBL CHROMagar Staph aureus** and **BBL CHROMagar MRSA II**, respectively. Bacteria other than *S. aureus* may utilize other chromogenic substrates resulting in blue, blue-green, or if no chromogenic substrates are

utilized, natural colored colonies. In **BBL CHROMagar MRSA II**, cefoxitin is added to render the medium selective for the detection of MRSA.
In order to easily differentiate both media from each other, titanium oxide is added to **BBL CHROMagar Staph aureus**. This insoluble compound renders **BBL CHROMagar Staph aureus** white and opaque while the **BBL CHROMagar MRSA II** is amber and transparent.

REAGENTS

Approximate Formulas* Per Liter of Purified Water

BBL CHROMagar Staph aureus		**BBL CHROMagar MRSA II**	
Chromopeptone	40.0 g	Chromopeptone	35.0 g
Sodium Chloride	25.0 g	Sodium Chloride	17.5 g
Chromogenic Mix	0.5 g	Chromogen Mix	0.5 g
Inhibitory Agents	0.07 g	Inhibitory Agents	7.52 g
Titanium Oxide	0,5 g	Cefoxitin	5.2 mg
Agar	14.0 g	Agar	14.0 g
pH: 6,8 +/- 0,2		pH: 7.0 +/- 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.
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.
Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.
After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.
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 their original wrapping and box at 2-8°C until time of inoculation. Minimize exposure (< 4h) to light both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see plate imprint or 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 2-8°C in the dark.

USER QUALITY CONTROL

Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions (for details, see **GENERAL INSTRUCTIONS FOR USE** document). The test strains mentioned in the Table below are recommended. Incubate **BBL CHROMagar Staph aureus** for 20-24 hours and **BBL CHROMagar MRSA II** for 20-22 hours, respectively, at 35 to 37° C aerobically, preferably in an inverted position, in the dark.

Strains	**Growth Results C-Staph aureus**	**Growth Results C-MRSA II**
Staphylococcus aureus ATCC™ 43300 (MRSA)	Growth of mauve colonies	Growth of mauve colonies
Staphylococcus aureus ATCC 29213 (MSSA)	Growth; mauve colonies	No growth
Staphylococcus saprophyticus ATCC 15305	Growth; green to blue-green colonies	No growth
Proteus mirabilis ATCC 12453	Inhibition (partial to complete)	No growth
Uninoculated	Opaque, white to cream	Light amber, transparent

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 clinical user refer to pertinent Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines for appropriate Quality Control practices.

PROCEDURE

Materials Provided

BBL CHROMagar Staph aureus / BBL CHROMagar MRSA II (Biplate), provided in divided 90 mm **Stacker** dishes. Microbiologically controlled.

Materials Required But Not Provided

Confirmatory test such as coagulase or *Staphylococcus* latex agglutination (e.g., **Staphyloslide**™) test reagents, quality control organisms, ancillary culture media and other laboratory equipment as required.

Specimen Types

Refer to appropriate texts or standards for details in specimen/sample collection and handling procedures.[9,10] The test can be performed on respiratory, lower gastrointestinal (= GI), skin and wound specimens, on anterior nares specimens for the screening of nasal colonization to aid in the prevention and control of *Staphylococcus aureus* and MRSA infections in healthcare settings and on positive blood culture bottles containing gram-positive cocci. See also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**.

Test Procedure

Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. As soon as possible after receipt of specimens in the laboratory, first inoculate a small area of **BBL CHROMagar Staph aureus** medium (opaque, whitish medium), then rotate the swab and inoculate a small area of **BBL CHROMagar MRSA II** medium (clear, amber medium). Afterwards, streak for isolation from the areas of first inoculation with a loop, first on **BBL CHROMagar Staph aureus**, and afterwards on **BBL CHROMagar MRSA II**. This sequence of inoculation must not be changed. Incubate aerobically at 35 – 37° C, preferably in an inverted manner, in the dark. For incubation times and interpretation, consult Tables 1 – 3.

RESULTS

Colonies of *Staphylococcus aureus* and MRSA, respectively, will appear mauve on **both chromogenic media of the biplate.** Other organisms will be inhibited or produce blue to blue/green, white or colorless colonies. Refer to Tables 1 - 3 for interpretation of results.

Principally, the following growth patterns can be obtained on **BBL CHROMagar Staph aureus / BBL CHROMagar MRSA II (Biplate)**:

BBL CHROMagar Staph aureus (opaque, white medium)	**BBL CHROMagar MRSA II** (transparent, amber medium)	**Interpretation**
Mauve colonies	No growth	***Staphylococcus aureus*** **(MSSA*)** detected
Mauve colonies	Mauve colonies	**MRSA** detected
No growth	No growth	*Staphylococcus aureus* (MSSA and MRSA) not detected
Non-mauve colonies	Non-mauve colonies	*Staphylococcus aureus* (MSSA or MRSA) not detected

*MSSA= methicillin-susceptible *Staphylococcus aureus*

Table 1: Interpretation of results for anterior nares specimens

Incubation: **CStaph aureus: 20-24 h** **CMRSAII: 20-26 h**	**Interpretation/Recommended Action**	
	BBL CHROMagar Staph aureus (opaque, white medium)	**BBL CHROMagar MRSA II** (transparent, amber medium)
Mauve colonies morphologically resembling	Positive – *Staphylococcus aureus* detected	Positive - MRSA detected

staphylococci*		
No mauve colonies detected	Negative – No *Staphylococcus aureus* detected	Negative - No MRSA detected

* See LIMITATIONS OF THE PROCEDURE

Table 2: Interpretation of results for positive blood culture bottles containing gram-positive cocci

Incubation: **CStaph aureus: 20-24 h** **CMRSAII: 18-28 h**	**Interpretation/Recommended Action**	
	BBL CHROMagar Staph aureus (opaque, white medium)	**BBL CHROMagar MRSA II** (transparent, amber medium)
Mauve colonies morphologically resembling staphylococci*	Positive – *Staphylococcus aureus* detected	Positive - MRSA detected
No mauve colonies detected	Negative – No Staphylococcus aureus detected	Negative - No MRSA detected

* See LIMITATIONS OF THE PROCEDURE

Table 3: Interpretation of results for throat, sputum, lower GI, skin and wound specimens

Incubation: **CStaph aureus: 20-24 h** **CMRSAII: 18-28 h**	**Interpretation/Recommended Action**	
	BBL CHROMagar Staph aureus (opaque, white medium)	**BBL CHROMagar MRSA II** (transparent, amber medium)
Mauve colonies morphologically resembling staphylococci*	Positive – *Staphylococcus aureus* detected	Positive - MRSA detected
No mauve colonies detected	Negative – No *Staphylococcus aureus* detected	Negative - No MRSA detected. Reincubate for additional 18 to 24 h to achieve a total incubation time of 36 – 52 hours)
Incubation: **CStaph aureus: 20-24 h** **CMRSAII: 36-52 h**	**Interpretation/Recommended Action**	
	BBL CHROMagar Staph aureus (opaque, white medium)	**BBL CHROMagar MRSA II** (transparent, amber medium)
Mauve colonies*	Interpretation beyond 24 h incubation is not recommended on this medium due to an increase in potential false positives. If incubation time is exceeded, mauve-colored colonies should be confirmed prior to reporting as *S. aureus*.	Perform direct confirmatory test (e.g., coagulase or *Staphylococcus* latex agglutination). If coagulase or *Staphylococcus* latex agglutination positive – MRSA detected If coagulase or *Staphylococcus* latex agglutination negative – No MRSA detected
No mauve colonies	Negative – No *Staphylococcus aureus* detected	Negative – No MRSA detected

* See LIMITATIONS OF THE PROCEDURE

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

Performance Results on BBL CHROMagar Staph aureus[12]

1. In a field trial conducted at a large US metropolitan hospital, 201 throat and sputum specimens from cystic fibrosis patients and 459 nasal specimens from other hospital patients were evaluated on **BBL CHROMagar Staph aureus**. **BBL CHROMagar Staph aureus** was compared to blood agar or Mannitol Salt Agar, with isolate confirmation by slide coagulase. *S. aureus* was recovered from 190 combined specimens. **BBL CHROMagar Staph aureus** detected 9 additional *S. aureus* positive cultures which were not recovered on conventional media. Four potential false positives were also observed on the **BBL CHROMagar Staph aureus** medium following 24 h incubation: two corynebacteria and two coagulase-negative staphylococci. **BBL CHROMagar Staph aureus** produced an overall sensitivity of 99.5% and a specificity of 99.2%.[11]

2. In a European study, one hundred sixty five (165) clinical specimens (76 wound specimens, 27 surgery specimens, 20 abscess specimens, and 42 specimens from miscellaneous sites) from a routine lab, consisting of 100 specimens shown to contain *S. aureus* by standard methods (= known positive specimens) and 65 known negative specimens, were streaked on **BBL CHROMagar Staph aureus**, Mannitol Salt Agar and Columbia Agar with 5% Sheep Blood. The specimen types are shown in Table 1. Plates were incubated for 20 to 24 hours at 35 to 37°C and were read for colonies suspicious of *S. aureus*. Tube coagulase tests were set up from all suspicious colonies on all three media. Of the 165 specimens, on **BBL CHROMagar Staph aureus**, 100 specimens yielded growth of S. aureus; on Mannitol Salt Agar, 91 yielded *S. aureus*; on Columbia Agar together with coagulase testing, 98 specimens were positive for *S. aureus*. There was one false positive on **BBL CHROMagar Staph aureus** that turned out to be *Streptococcus agalactiae*. Upon restreaking the strain on **BBL CHROMagar Staph aureus**, the colonies were violet rather than rose to mauve.
Among the known negative specimens, there were 5 cultures with violet or lilac colonies which were similar to *S. aureus* in color. However, they could be easily differentiated from *S. aureus* colonies (=rose to mauve).
The sensitivities of **BBL CHROMagar Staph aureus** (based on rose to mauve colony color), Mannitol Salt Agar (based on colonies surrounded by yellow medium), and Columbia Agar (growth of typical *S. aureus* colonies together with coagulase testing) were 100%, 91%, and 98%. The specificity of **BBL CHROMagar Staph aureus** was 98.5%.[11]
For details, consult Instructions for Use of **BBL CHROMagar Staph aureus** (PA-257074).

Performance Results on BBL CHROMagar MRSA II

A combined overall total of 5051 specimens (consisting of 1446 respiratory, 694 gastrointestinal, 1275 skin, 948 wound specimens and 688 blood cultures positive for Gram positive cocci) were evaluated comparing the recovery of MRSA on traditional culture plates (e.g., Tryptic Soy Agar with 5% Sheep Blood, Columbia Agar with 5% Sheep Blood, or CNA [colistin nalidixic acid agar]) to **BBL CHROMagar MRSA II** plates.

Overall recovery of MRSA on **BBL CHROMagar MRSA II** was higher at 95.6% (744/778) compared to a recovery of 79.8% (621/778) on traditional culture plates for all specimen types combined (respiratory, lower GI, skin, wound and positive blood culture bottles containing gram-positive cocci). At the 18-28 h reading, there were 2 false positive mauve colonies observed on **BBL CHROMagar MRSA II**, for a specificity of 99.9% (4271/4273). Using colony color at the 18-28 h reading for **BBL CHROMagar MRSA II**, and confirming all mauve colonies with confirmatory testing at the 36-52 h reading, the combined overall agreement of **BBL CHROMagar MRSA II** compared to the cefoxitin disk diffusion test for all specimen types was 99.3% (5015/5051).11,12 For details, consult Instructions for Use of **BBL CHROMagar MRSA II** (PA-275434).

LIMITATIONS OF THE PROCEDURE

General information:

- Minimize exposure of **BBL CHROMagar Staph aureus /BBL CHROMagar MRSA II (Biplate)** to light (<4 h) both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates.
- Incubation in CO_2 is not recommended and may result in false negative cultures.
- A heavy bacterial load and/or some specimens may produce nonspecific coloring of the primary streak area of the media. This could result in the medium exhibiting mauve, purple, green or blue coloration or a slight haze on top of the medium, but lacking distinct colonies. Non-specific coloring of the medium should be interpreted as negative.
- A single negative result should not be used as the sole basis for diagnosis, treatment, or management decisions. Concomitant cultures may be necessary for organism identification, susceptibility testing or epidemiological typing.
- Before using **BBL CHROMagar Staph aureus / BBL CHROMagar MRSA II (Biplate)** for the first time, training on the typical colony appearance of *S. aureus* and MRSA with

defined strains; e.g., the strains mentioned under **User Quality Control**, is recommended.

BBL CHROMagar Staph aureus:

- Occasionally some strains of staphylococci, other than *S. aureus*, such as: *S. cohnii*, *S. intermedius*, and *S. schleiferi*, as well as corynebacteria and yeasts, may produce mauve-colored colonies at 24 h.[11] Differentiation of *S. aureus* from non-*S. aureus* can be accomplished by coagulase, other biochemicals or Gram stain. Resistant gram-negative bacilli, which typically appear as small blue colonies, may also break through.
- Incubation of **BBL CHROMagar Staph aureus** beyond 24 h is not recommended due to an increase in potential false positives. If incubation time is exceeded, mauve-colored colonies should be confirmed prior to reporting as *S. aureus*.
- Incubation less than the recommended 20 h may result in a lower percentage of correct results being obtained.
- Due to the natural golden pigment of some *S. aureus* strains, colony color may appear orange-mauve.

BBL CHROMagar MRSA II:

- Incubation time beyond 36 - 52 h is not recommended.
- For anterior nares specimens, performance of **BBL CHROMagar MRSA II** has been optimized for incubation for 20-26 h at 35-37°C. Lower incubation temperatures (<35° C) and/or shorter incubation times (<20 h) may reduce the sensitivity of **BBL CHROMagar MRSA II**. Note that frequent opening of incubator doors may reduce the incubator temperature. It is therefore recommended to reduce opening of the incubator doors to a minimum and to keep the opening periods as short a possible.
- After 24 h or longer incubation, some strains of *Chryseobacterium meningosepticum*, *Corynebacterium jeikeium*, *Enterococcus faecalis* (VRE), *Rhodococcus equi*, and *Bacillus cereus* may produce mauve-colored colonies. If desired, a gram stain may be performed.
- After 24 h or longer incubation, *Staphylococcus simulans*, *S. epidermidis,* and methicillin-susceptible *Staphylococcus aureus* rarely may also produce mauve-colored colonies. If MRSA is not suspected, a coagulase test and antimicrobial susceptibility test (AST) may be performed.
- Rare strains of MRSA have demonstrated sensitivity to the **BBL CHROMagar MRSA II** base. This sensitivity is unrelated to methicillin resistance, but is due to a component in the base. As a result, these strains may appear as falsely susceptible to methicillin.
- There exist rare strains of MRSA that may produce non-mauve colonies on **BBL CHROMagar MRSA II**. If MRSA is suspected, subculture non-mauve colonies for further identification and susceptibility testing as necessary.
- *mec*A-negative *S. aureus* may grow if the oxacillin or cefoxitin MICs are at or near the resistant breakpoint.
- Resistance mechanisms other than *mecA* (i.e. borderline oxacillin-resistant *Staphylococcus aureus*-BORSA, and modified *Staphylococcus aureus*-MODSA), have not been extensively evaluated with the CMRSA II, therefore the performance of CMRSA II with such resistance mechanisms is unknown.
- Because the isolation of MRSA is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.

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PACKAGING/AVAILABILITY

BD BBL™ CHROMagar™ Staph aureus /BBL™ CHROMagar™ MRSA II (Biplate)

Cat. No.	Description
REF 257585	Ready-to-use Plated Media, cpu 120
REF 257699	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
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/