BD BBL™ CHROMagar™ MRSA*

INTENDED USE

BBL CHROMagar MRSA is a selective and differential medium for the qualitative direct detection of colonization by methicillin resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test is performed on anterior nares swab specimens, from patients and healthcare workers to screen for MRSA colonization. **BBL CHROMagar MRSA** is not intended to diagnose MRSA infection nor to guide or monitor treatment for infections.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

MRSA are a major cause of nosocomial and life-threatening infections. Infections with MRSA have been associated with a significantly higher morbidity, mortality and costs than methicillin-susceptible S. *aureus* (MSSA).[1]

The prevalence of MRSA infection has increased dramatically in medical institutional settings, and the carriage rate of MRSA is rising in the community.[2] Recent publications suggest that the population at large has *S. aureus* colonization rates ranging between 25 and 30%.[3]

Resistance rates have steadily increased in the past fifteen years, and recent NNIS (National Nosocomial Infections Surveillance) data indicates that, in the intensive care patient setting, the proportion of MRSA among *S. aureus* infections was as high as 60% in 2003.[4]

To control the transmission of MRSA, the Society for Healthcare Epidemiology of America (SHEA) has recommended guidelines, which include an active surveillance program to identify potential reservoirs and a rigorous infection control program to control the spread of MRSA.[1]

BBL CHROMagar MRSA permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and cefoxitin. MRSA strains will grow in the presence of cefoxitin[5] and produce rose to mauve-colored colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of gram-negative organisms, yeast and some gram-positive cocci. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in blue to blue/green colored colonies or, if no chromogenic substrates are utilized, the colonies appear as white or colorless.

BBL CHROMagar MRSA was developed by A. Rambach and BD. This product utilizes **BBL CHROMagar** Staph aureus, which was developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

REAGENTS

BBL CHROMagar MRSA

Formula* Per Liter Purified Water

Chromopeptone	40.0 g
Sodium Chloride	25.0
Chromogen Mix	0.5
Inbibitory agents	0.07
Cefoxitin	0.006
Agar	14.0

pH 6.8 ± 0.3

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

*** US Patent Pending**

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" [6-9] 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.

For details on aseptic handling procedures, biohazards, and disposal of used product consult **GENERAL INSTRUCTIONS FOR USE** document.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping and cardboard box until just prior to use. Avoid freezing, overheating and exposure to light before and during incubation as light may destroy the chromogens. 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 in the dark.

USER QUALITY CONTROL

Examine plates for signs of deterioration as described under **PRECAUTIONS**. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. To determine the inhibitory capacity of the medium, S. *aureus* ATCC™ 25923 should be inoculated at a concentration of 10^4- 10^5 CFU/plate.[10] To determine the nutritive capacity of the medium, S. *aureus* ATCC 43300 should be inoculated at a concentration of 10^3 -10^4 CFU/plate.[10]

Incubate aerobically at 35 to 37° C for **24 ± 4 hours**. Do not incubate in an atmosphere supplemented with carbon dioxide.

Strains	Growth Results
Staphylococcus aureus ATCC™ 25923 (MSSA)	No growth
Staphylococcus aureus ATCC 43300 (MRSA)	Growth with moderately sized rose to mauve colonies
Uninoculated	Light beige, transparent

PROCEDURE

Materials Provided

BBL CHROMagar MRSA (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Required But Not Provided

Ancillary culture media, coagulase test reagents, quality control organisms and other laboratory equipment as required.

Specimen Types

This medium has been evaluated for performance with anterior nares specimens. Until now, only a limited number of clinical specimens from various body sites has also been tested (see **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Use of transport devices approved for the collection of such specimens is recommended. Follow the transport device manufacturer's recommended procedures. The user may also refer to appropriate texts for details of specimen collection and handling procedures.[11,12]

Test Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a **BBL CHROMagar MRSA** plate and streak for isolation, using a loop.

Incubate plates aerobically at 35-37°C for **24 ± 4 hours** in an inverted position. If no rose to mauve colonies are recovered, reincubate for an additional 24 h. Do not incubate in an

atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation (> 4 hours) as light may destroy the chromogens. Exposure to light is permissible after colony color develops.

Important note: It has been determined that low incubation temperature (<35° C) and/or short incubation time (<20 hours) can significantly reduce the sensitivity of **BBL CHROMagar MRSA** in obtaining results after 1 day reading of the plates. Therefore, it is important that the ideal incubation temperature of 36° C (acceptable range: 35 to 37° C) is maintained throughout the incubation time (not less than 20 hours; ideal is 22 hours for reading first day results). Repeated opening of incubator doors will reduce the actual 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. If this cannot be achieved, it is recommended to incubate **BBL CHROMagar MRSA** in a dedicated incubator.

Results
Read plates against a white background. Colonies of MRSA will appear rose to mauve on the **BBL CHROMagar MRSA** medium. Other organisms (non-MRSA) will be inhibited or produce colorless, white, blue or blue/green colonies. Refer to Table 1 for interpretation of results.

Table 1

24 h Incubation	**Interpretation/Recommended Action**
Rose to mauve colonies morphologically resembling staphylococci*	MRSA detected, report MRSA nasal colonization
No rose to mauve colonies	No result available, reincubate 24 additional hours

48 h Incubation	**Recommended Action**	**Interpretation**
Rose to mauve colonies	Perform coagulase testing	If coagulase positive – MRSA detected, report MRSA. If coagulase negative – report no MRSA detected
No rose to mauve colonies	N/A	Report no MRSA detected

*Staphylococci typically produce moderately sized smooth rose to mauve colonies on **BBL CHROMagar MRSA** medium. Mauve colonies which are very small to pinpoint are most often gram positive rods, usually corynebacteria. If morphology is unclear, confirmatory tests such as coagulase may be used to confirm identification at 48 h.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BBL CHROMagar MRSA is used for the qualitative direct detection, isolation, and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal surveillance specimens at 24 h incubation without confirmatory testing or at 48 h incubation with a confirmatory coagulase test (see **Limitations of the Procedure**).

Performance Characteristics[13]
Performance Evaluations

1. **BBL** CHROMagar MRSA was evaluated at four geographically diverse US hospitals with fresh prospective surveillance specimens of the anterior nares. A total of 1974 surveillance nares specimens specimens were evaluated, comparing the recovery of MRSA on **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** reference plates to **CHROMagar MRSA** plates. *S. aureus* recovered on TSA II were tested by a microbroth dilution Oxacillin MIC method, and an Oxacillin Screen Agar method, as well as three additional susceptibility test methods (see next section). Oxacillin MIC results followed NCCLS interpretive criteria, with MSSA ≤2 µg/ml and MRSA ≥4 µg/ml. Oxacillin Screen Agar was interpreted using manufacturer's instructions which included the presence of any colony growth as representative of MRSA. **CHROMagar MRSA** was interpreted as positive for MRSA at 24 h based on detection of mauve colony color (alone), or at 48 h based on detection of mauve colonies with confirmation as *S. aureus* by a coagulase test. Overall recovery of MRSA on **CHROMagar MRSA** was higher at 95% (126), compared to a recovery of 89% (117) on TSA II. The accuracy of identification of MRSA was compared to the Oxacillin MIC microbroth dilution method and the Oxacillin Screen Agar method. At the 24 h reading, there were 6 false positives where mauve colonies were observed on **CHROMagar MRSA** (2 *S. epidermidi*s, 2 *S. haemolyticu*s, and 2 *Corynebacteriu*m). Using colony color alone at the 24

h reading for **CHROMagar MRSA**, and confirming all mauve colonies with coagulase at the 48 h reading, the overall agreement of the **CHROMagar MRSA** test to the Oxacillin MIC test was 96% (312/325). Overall category agreement of **CHROMagar MRSA** to Oxacillin Screen Agar was 96% (312/325). Positive percent MRSA agreement and negative percent MSSA agreement of **CHROMagar MRSA** compared to these reference methods is shown in the following Tables 2 to 5:

Table 2: Performance of BBL CHROMagar MRSA (24 h mauve / 48 h with coagulase combined final result) versus Oxacillin MIC Reference Result:

		TSA II Result			
		Growth of *S. aureus*			
		Oxacillin MIC Reference Result			
CHROMagar MRSA Result	**MRSA Identification**	**MRSA**	**MSSA**	**No growth of *S. aureus***	**Total**
Mauve	Mauve at 24 h or mauve and coag pos at 48 h	111	7	21*	139
	Coag neg 48 h	0	3	68**	71
Not mauve / no growth	N/A	6	198	1560	1764
Total		117	208	1649	1974

*Of 21 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **BBL CHROMagar MRSA**: 15 were confirmed as MRSA by positive PBP2' latex test results; 4 were coagulase-negative staphylococci, and 2 were Gram positive rods.
** Of 68 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **BBL CHROMagar MRSA** at 48 h: 45 were confirmed as coagulase-negative staphylococci; and 23 were Gram positive rods and other organisms.

Table 3

CHROMagar MRSA vs. Oxacillin MIC	
Sensitivity (95%CI)	**Specificity (95%CI)**
94.9% (111/117) (89.3%; 98.1%)	96.6% (201/208) (93.2%; 98.6%)

Table 4: Performance of BBL CHROMagar MRSA (24 h mauve / 48 h with coagulase combined final result) versus Oxacillin Screen Agar Reference Result:

		TSA II Result			
		Growth of *S. aureus*			
		Oxacillin Screen Agar Reference Result			
CHROMagar MRSA Result	**MRSA Identification**	**MRSA**	**MSSA**	**No growth of *S. aureus***	**Total**
Mauve	Mauve at 24 h or mauve and coag pos at 48 h	110	7	21*	138
	Coag neg 48 h	0	3	68**	71
Not mauve / no growth	N/A	6	199	1560	1765
Total		116	209	1649	1974

*Of 21 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **BBL CHROMagar MRSA**: 15 were confirmed as MRSA by positive PBP2' latex test results; 4 were coagulase-negative staphylococci, and 2 were Gram positive rods.
** Of 68 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **BBL CHROMagar MRSA** at 48 h: 45 were confirmed as coagulase-negative staphylococci; and 23 were Gram positive rods and other organisms.

Table 5

CHROMagar MRSA vs. Oxacillin Screen Agar	
Sensitivity (95%CI)	**Specificity (95%CI)**
94.8% (110/116) (89.1%; 98.1%)	96.7% (202/209) (93.2%; 98.6%)

These studies also compared **BBL CHROMagar MRSA** to other test methods for identifying MRSA: the PBP 2' Latex Agglutination Test, a cefoxitin (30 µg) disk diffusion test, and PCR detection of the *mecA* gene. The cefoxitin disk diffusion testing followed recent NCCLS interpretive criteria (zone size of ≤19 mm as MRSA, or ≥ 20 mm as MSSA).[5] PBP 2' and PCR methods followed labeling instructions for interpretation. Percent agreement compared to these additional methods is shown in Table 6 for the MRSA and MSSA isolates. Total number of isolates tested differs between methods due to differences in individual method completion or compliance/evaluability rates.

Table 6

CHROMagar MRSA vs. Cefoxitin Disc Diffusion		CHROMagar MRSA vs. PBP 2' Latex Agglutination		CHROMagar MRSA vs. PCR (*mec*A)	
% Agreement of MRSA	**% Agreement of MSSA**	**% Agreement of MRSA**	**% Agreement of MSSA**	**% Agreement of MRSA**	**% Agreement of MSSA**
94.9% (112/118) (89.3%; 98.1%)	98% (200/204) (95.1%; 99.5%)	93.5% (115/123) (87.6%; 97.2%)	98.5% (198/201) (95.7%; 99.7%)	95.7% (111/116) (90.2%; 98.6%)	97% (196/202) (93.6%; 98.9%)

2. In a European study, surveillance specimens and other clinical specimens were tested. For routine laboratory investigation of MRSA detection, the specimens were plated on Columbia CNA Agar with 5% Sheep Blood, and specimens suspicious for *S. aureus* were subjected to PCR for *S. aureus* and MRSA. The specimens were kept refrigerated after processing. Directly after the PCR result was available they were plated on **CHROMagar MRSA** and onto Columbia CNA with 5% Sheep Blood. Plates were incubated aerobically at 36 +/- 1° C and were read after 22 to 24 hours incubation. In case of no growth of colonies suspicious for *S. aureus* on one or both media, plates were re-incubated for additional 20 to 24 hours. For confirmation, rose to mauve colonies from **CHROMagar MRSA** and colonies suspicious for *S. aureus* on Columbia CNA Agar were subjected to a tube coagulase test and were tested for growth on Oxacillin Screen Agar and for cefoxitin resistance with a disc diffusion test, using the NCCLS criteria (zone sizes of </= 19 mm indicate MRSA).[5]
   PCR-positive surveillance specimens (n= 50) included: 37 nasal swabs, 1 throat/nose swab, 9 throat swabs, and 3 skin swabs.
   Other PCR-positive specimens (n= 30) included 2 abscess and 3 surgery specimens, 23 wound swabs, and 2 ulcus specimens.
   PCR-negative specimens (n= 55) included 3 abscess specimens, 9 skin swabs, 1 decubitus swab, 15 nasal swabs, 10 throat swabs, 5 perineal swabs, 1 puncture specimen, 3 catheter swabs, 1 tracheal secretions specimen, and 7 wound swabs.
   Altogether, 135 specimens were tested.

   All 80 PCR positive specimens yielded growth of rose to mauve colonies on **CHROMagar MRSA** and colonies suspicious of *S. aureus* on Columbia CNA Agar with 5% Sheep Blood after 22 to 24 hours, while the 55 PCR negative specimens did not show the respective growth on the two media after 22 to 24 and after 42 to 48 hours. Two isolates from the PCR negative specimens obtained on Columbia CNA but not on **CHROMagar MRSA** were confirmed as *S. aureus* by a positive coagulase test; these isolates did not grow on Oxacillin Screen Agar and were cefoxitin susceptible (zone size 30 mm) and did not produce rose to mauve colonies on **CHROMagar MRSA**. Another isolate from a PCR negative specimen produced violet colonies on **CHROMagar MRSA** that could be differentiated by colony color from the rose to mauve coloration of *S. aureus*.
   All 80 MRSA positive specimens produced growth on Oxacillin Screen Agar from both **CHROMagar MRSA** and Columbia CNA Agar with 5% Sheep Blood.

In the cefoxitin disc test, two isolates showed susceptibility both when subcultured from **CHROMagar MRSA** and Columbia CNA Agar with 5% Sheep Blood, and four strains showed resistance when subcultured from **CHROMagar MRSA** but susceptibility when subcultured from Columbia CNA Agar with 5% Sheep Blood. All other isolates showed resistance from both CHROMagar MRSA and Columbia CNA Agar.
Sensitivity and specificity as compared to PCR and Oxacillin Screen Agar was 100%.
Sensitivity as compared to the cefoxitin disc test was 91.4%.

Challenge Testing
Testing of twenty (20) challenge strains of S. *aureus* was conducted at three of the US clinical sites. In this panel, 9 were heterogeneous resistant MRSA, 5 were homogeneous resistant MRSA, and 6 were MSSA. Individual site and combined site sensitivities were aII 100%, and site and overall specificities were 100%.

Expression of Resistance
BBL CHROMagar MRSA was evaluated for its ability to detect heterogeneous and homogeneous strains. MRSA can be homogeneously or heterogeneously resistant. Heterogeneous strains may have as few as 1 in 1 million cells expressing resistance, making detection by conventional antimicrobial susceptibility tests difficult.[14] Fifteen test strains, representing 10 heterogeneous and 5 homogeneous MRSA, were evaluated for recovery and colony counts on **BBL CHROMagar MRSA** compared to a nonselective medium, TSA II with 5% sheep blood. Both **BBL CHROMagar MRSA** and TSA II recovered all 15 strains. **BBL CHROMagar MRSA** colony counts ranged from 64-99% for heterogeneous strains and 71-100% for homogeneous strains compared to the TSA II. These results support that **BBL CHROMagar MRSA** is able to detect both homogeneous and heterogeneous strains.[14]

Interference Studv
Eight commonly used medicinal substances, human blood and five types of specimen transport devices, were evaluated for potential interference of the chromogenic reaction on the **BBL CHROMagar** MRSA medium. At a 10% concentration, a nasal spray containing phenylephrine hydrochloride demonstrated antibacterial activity on **BBL CHROMagar** MRSA, as well as on the nonselective control, TSA II with 5% sheep blood. No other substance or device tested interfered with the performance of the **BBL CHROMagar** MRSA medium.[13]

Expected Values
In the external performance evaluation of **CHROMagar MRSA** (see **Performance Characteristics**), the overall prevalence of *S. aureus* colonization was 17.2% (340/1974), as detected by either the **CHROMagar MRSA** or **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** plates. The overall prevalence of (non-duplicate patient) MRSA-positive specimens was 6.7% (132/1974), or about 39% (132/340) of all *S. aureu*s. The TSA II plate MRSA-colonization detection rate was 6.5% (117/1974), while the **CHROMagar MRSA** rate of MRSA-colonization was 7.0% (126/1974). The colonization rates may vary within different countries and population groups.[3,4]

Limitations of the Procedure
Minimize exposure of **BBL CHROMagar MRSA** to light both 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.

Surveillance testing determines the colonization status at a given time and could vary depending on patient treatment (e.g. decolonization regime), patient status (e.g. not actively shedding MRSA) or exposure to high risk environments (e.g. contact with MRSA carrier, prolonged hospitalization). Monitoring colonization status should be done according to hospital policies. Results from **CHROMagar MRSA** should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions.
This medium can be used to identify patients for isolation or removal from isolation to control nosocomial transmission of MRSA. A **CHROMagar MRSA** negative result following a previous

positive test result may indicate treatment eradication success or may occur due to intermittent shedding.

If clinical specimens are examined, it is necessary to inoculate additional media with these specimens, especially a nonselective blood agar plate (e.g., **BD Columbia Agar with 5% Sheep Blood**) and, to improve the recovery of Gram positive organisms involved in the infection, **BD Columbia CNA Agar with 5% Sheep Blood**.
Certain *Enterococcus* strains are resistant to the inhibitory agents included in **BBL CHROMagar MRSA**. Rarely, this may result in overgrowth of blue to blue-green colonies, making detection of MRSA difficult. If strong growth of blue-green colonies is observed, it is recommended to compare the growth obtained on **BBL CHROMagar MRSA** with the growth on the blood agar plate for the presence of *S. aureus*.

Strictly follow the incubation times and temperatures mentioned in **PROCEDURE - Test Procedure**.

At 48 h occasional strains of coagulase-negative staphylococci (such as, S. *epidermidis,* S. *cohnii,* S. *intermedius,* S. *haemolyticus,* S. *capitis,* S. *hominis* and S. *schleiferi), Acinetobacter* sp., corynebacteria and yeast may produce mauve-colored colonies requiring a confirmatory coagulase test for confirmation of MRSA. This may also occur at a much lower rate at 24 h. In clinical studies with surveillance specimens, approximately 5% (6/120) of the mauve colored colonies detected at 24 h were coagulase-negative staphylococci and/or corynebacteria on the **BBL CHROMagar MRSA** medium. If desired, a Gram stain and/or a coagulase test may be performed at 24 h on mauve-colored colonies to increase specificity.

If the oxacillin or cefoxitin MICs of an isolate are at or near the resistant breakpoint, *mec*A-negative S. *aureus* (borderline resistant *S. aureus* or BORSA) may grow.

Incubation in 5% CO_2 is not recommended and may result in false negative cultures.

Use of phenylephrine hydrochloride, a component of some nasal sprays, at a concentration of ≥10% shows an inbibitory effect on organism growth that is unrelated to medium performance.

Rare strains of MRSA have demonstrated sensitivity to the **BBL CHROMagar MRSA** 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.
CHROMagar MRSA is not intended to detect *S. aureus* other than MRSA or other *Staphylococcus* species.

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

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PACKAGING/AVAILABILITY

BD BBL CHROMagar MRSA

REF 257308 Ready-to-use Plated Media, cpu 20

REF 257333 Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
BD Diagnostic Systems
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

BD Diagnostic Systems Europe
Becton Dickinson France SA
11 rue Aristide Bergès
38800 Le Pont de Claix/France
Tel: +33-476 68 3636 Fax: +33-476 68 3292 http://www.bd.com