



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

**BBL™ CHROMagar™ MRSA II** is a selective and differential chromogenic medium for the qualitative direct detection of nasal colonization by methicillin-resistant *Staphylococcus aureus* (MRSA).

### II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the cultures listed below.
  - Streak the plates for isolation. For *Enterococcus faecalis* ATCC™ 29212 and *Staphylococcus aureus* ATCC 25923 and 29213, dilute cultures to yield 10<sup>4</sup>-10<sup>5</sup> CFU/plate. For *Staphylococcus aureus* ATCC 33591 and 43300 dilute cultures to yield 10<sup>3</sup>-10<sup>4</sup> CFU/plate.
  - Incubate plates at 35 ± 2°C in an aerobic atmosphere.  
**NOTE:** Minimize exposure to light before and during incubation.
  - Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 20 – 26 h for recovery, colony size, and color.
- Expected Results

Organisms	ATCC™	Recovery	Colony Color
<i>Enterococcus faecalis</i>	29212	Inhibition (partial to complete)	No growth or non-mauve colonies
<i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	No growth or non-mauve colonies
* <i>Staphylococcus aureus</i>	29213	Inhibition (partial to complete)	No growth or non-mauve colonies
<i>Staphylococcus aureus</i>	33591	Growth	Mauve
* <i>Staphylococcus aureus</i>	43300	Growth	Mauve

\*Recommended organism strain for User Quality Control. Direct inoculation may be used for User Quality Control.<sup>1</sup>

**NOTE:** Before using **BBL CHROMagar MRSA II** for the first time, training on the typical colony appearance of MRSA with defined strains is recommended.

### III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification 7.0 ± 0.2.
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

**BBL™ CHROMagar™ MRSA II** is a selective and differential chromogenic medium for the qualitative direct detection of nasal 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 to screen for MRSA colonization.

**BBL CHROMagar MRSA II** is not intended to diagnose, guide or monitor treatment for MRSA infections. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary for organism identification, susceptibility testing or epidemiological typing.

### V SUMMARY AND EXPLANATION

MRSA are 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).<sup>2</sup> Selection of these organisms has been greatest in the healthcare setting; however, MRSA has also become more prevalent in the community.<sup>3</sup> To control the transmission of MRSA, the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) have recommended guidelines, which include monitoring MRSA transmission, infection control programs to control transmission and implementation of active surveillance testing in hospital populations and areas where MRSA is not effectively controlled.<sup>2</sup>

**BBL CHROMagar MRSA II** is a selective and differential medium, which incorporates cefoxitin for the detection of MRSA from anterior nares specimens.

**BBL CHROMagar MRSA II** is a modified version of the existing formulation of **BBL CHROMagar MRSA** developed by A. Rambach and BD and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

### VI PRINCIPLES OF THE PROCEDURE

**BBL CHROMagar MRSA II** medium 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<sup>4</sup> and produce mauve colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of gram-negative organisms, yeast and some other gram-positive cocci. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in the growth of colonies that are not mauve.

## VII REAGENTS

### BBL CHROMagar MRSA II

Approximate Formula\* Per Liter Purified Water

Chromopeptone .....	35.0	g
Chromogen Mix .....	0.5	g
Sodium Chloride .....	17.5	g
Inhibitory Agents .....	7.52	g
Cefoxitin .....	5.2	mg
Agar .....	14.0	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 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. Protect from light during drying. See storage instructions.

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. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in their original sleeve wrapping and box at 2 – 8°C until time of inoculation. Prolonged exposure to light (> 4 h) may result in reduced recovery and/or coloration of the QC strains or patient isolates. Plates may be used until the expiration date. Avoid freezing and overheating.

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

## VIII SPECIMEN COLLECTION AND HANDLING

This device has been evaluated for performance with anterior nares specimens. Use of transport devices approved for the collection of microbiological clinical 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.<sup>9,10</sup>

## IX PROCEDURE

**Material Provided:** BBL CHROMagar MRSA II

**Materials Required But Not Provided:** Quality control organisms, ancillary culture media and other laboratory equipment as required.

**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 in the dark before inoculation.

As soon as possible after receipt in the laboratory, inoculate the specimen onto a **BBL CHROMagar MRSA II** plate and streak for isolation. Incubate plates aerobically at 35 – 37°C for 20 – 26 h in an inverted position. Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation. Exposure to light is permissible after colony color develops.

**User Quality Control:** See "Quality Control Procedures."

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.

Before using **BBL CHROMagar MRSA II** for the first time, training on the typical colony appearance of MRSA with defined strains (e.g., the strains mentioned under "Quality Control Procedures") is recommended.

## X RESULTS

Read plates against a white background. Colonies of MRSA will appear mauve on the **BBL CHROMagar MRSA II** medium. Refer to Table 1 for interpretation of results.

**Table 1: Interpretation of results for anterior nares specimens**

20-26 h Incubation	Interpretation/Recommended Action
Mauve colonies morphologically resembling staphylococci	Positive - MRSA detected
Non-mauve colonies detected*	Negative - No MRSA detected
No growth	Negative. A negative result does not preclude MRSA nasal colonization. If MRSA is suspected, e.g., based on patient history, an alternate method for confirming MRSA should be used.

\* Certain MRSA 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.

## XI LIMITATIONS OF THE PROCEDURE

- A negative result should not be used as the sole basis for diagnosis, treatment, or management decisions. A negative result does not preclude MRSA nasal colonization.
- Minimize exposure (< 4 h) of **BBL CHROMagar MRSA II** to light both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates.
- Keep plates within the original sleeve wrapping and box for the entire storage period.
- Performance of **BBL CHROMagar MRSA II** has been optimized for incubation at 35 – 37°C for 20 – 26 h. Lower incubation temperatures (< 35°C) and/or shorter incubation times (< 20 h) may reduce the sensitivity of **BBL CHROMagar MRSA II**.
- MRSA concentrations of lower than 10<sup>6</sup> CFU/mL may yield false negative results on **BBL CHROMagar MRSA II** (refer to Sensitivity - Analytical Reactivity).

- At 24 h, 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.
- At 24 h, *Staphylococcus simulans*, *S. epidermidis*, and methicillin-susceptible *Staphylococcus aureus* may also produce mauve-colored colonies. If MRSA is not suspected, a coagulase test and antimicrobial susceptibility test (AST) may be performed.
- Nasal sprays containing fluticasone propionate, azelastine hydrochloride and oxymetazoline hydrochloride as well as OTC throat drops containing menthol demonstrated antibacterial activity.
- *mecA*-negative *S. aureus* demonstrated variable results on this medium and may grow if the oxacillin or *mecA* mediated cefoxitin MICs are at or near the resistant breakpoint.
- In the event of mixed infection, the accuracy of this device for detecting MRSA in the presence of other bacteria at a concentration higher than 1 x10<sup>9</sup> CFU/mL has not been established and is therefore unknown.
- Resistance mechanisms other than *mecA* (i.e., borderline oxacillin-resistant *Staphylococcus aureus*-BORSA, and modified *Staphylococcus aureus*-MODSA), have not been extensively evaluated with **BBL CHROMagar MRSA II**, therefore the performance of **BBL CHROMagar MRSA II** with such resistance mechanisms is unknown.
- The growth requirements of certain strains of MRSA can lead to their partial or complete inhibition in culture.
- 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 **BBL CHROMagar MRSA II** should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. Results should not be used to guide or monitor treatment of MRSA infections. This device can be used to identify patients for isolation or removal from isolation to control nosocomial transmission of MRSA.
- A **BBL CHROMagar MRSA II** result of MRSA not detected following a previous test with MRSA detected may indicate treatment eradication success or may occur due to intermittent shedding. A recent study demonstrated that a negative culture, following three negative weekly surveillance cultures, can predict clearance of MRSA colonization in most (94%) colonized patients.<sup>11</sup>
- Incubation in CO<sub>2</sub> 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 quadrant of the medium. 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 not be interpreted as positive.
- Pediatric samples were not extensively analyzed during the clinical investigation; therefore, the performance of this assay with pediatric samples 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.

## XII EXPECTED VALUES

The prevalence of MRSA infection has increased dramatically in medical institutional settings, and the carriage rate of MRSA is rising in the community. Recent publications suggest that the population at large has *S. aureus* colonization rates ranging from 25 to 30%.<sup>12</sup> Data from the NNIS (National Nosocomial Infections Surveillance System) indicate that in the intensive care patient setting, the proportion of MRSA among *S. aureus* infections has increased to 59.5 – 64.4%.<sup>12,13</sup> In the clinical evaluation described below, the overall prevalence of MRSA was 13.6%, or 49.8% (162/325) of all *S. aureus* isolates tested.

## XIII PERFORMANCE CHARACTERISTICS

### Clinical studies

**BBL CHROMagar MRSA II** was evaluated at three geographically diverse clinical laboratories with surveillance specimens of the anterior nares. Specimens were evaluated by comparing the recovery of MRSA on **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** plates and each site's routine procedure for identification of *S. aureus* (Traditional Culture) to **BBL CHROMagar MRSA II** plates. The routine procedure for two sites included staphylococcal latex agglutination testing and the third site included coagulase testing. All *S. aureus* recovered were tested for *mecA* mediated oxacillin resistance by the cefoxitin disk diffusion test. Cefoxitin disk (30 µg) diffusion test results followed CLSI methods and interpretive criteria.<sup>4,14</sup> **BBL CHROMagar MRSA II** was interpreted as positive for MRSA at 20 – 26 h based on detection of mauve colonies.

Table 2: **BBL CHROMagar MRSA II (CMRSA II) Performance vs. Cefoxitin Disk**

CMRSA II Result	Cefoxitin Disk		Total
	MRSA	Not MRSA	
MRSA	149	1	150
Not MRSA	13	1024	1037
	162	1025	1187
Reference Method: Cefoxitin Disk			
Positive Percent Agreement: 92% (86.7%, 95.7%)			
Negative Percent Agreement: 99.9% (99.5%, 100%)			

The positive percent agreement and negative percent agreement of **BBL CHROMagar MRSA II** at 20 – 26 h was 92% and 99.9%, respectively, using the cefoxitin disk result as reference (Table 3).

**Table 3: BBL CHROMagar MRSA II Performance vs. Cefoxitin Disk**

Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
92% (149/162) (86.7%,95.7%)	99.9% (1024/1025) (99.5%,100%)

With combined data from two clinical trial sites, the positive percent agreement of **BBL CHROMagar MRSA II** compared to Traditional Culture was 92% at 20 – 26 h and the negative percent agreement was 98.8% (Table 4).

**Table 4: BBL CHROMagar MRSA II Performance vs. Traditional Culture at Two Clinical Trial Sites**

CMRSA II Result	Traditional Culture		Total
	MRSA	Not MRSA	
MRSA	92	9*	101
Not MRSA	8	760	768
	100	769	869
Reference Method: Traditional Culture			
Positive Percent Agreement: 92% (84.8%, 96.5%)			
Negative Percent Agreement: 98.8% (97.8%, 99.5%)			

\* Nine samples that were positive on **BBL CHROMagar MRSA II** and negative by Traditional Culture were confirmed as MRSA by cefoxitin disk diffusion testing.

At the third clinical trial site, the positive percent agreement of **BBL CHROMagar MRSA II** compared to Traditional Culture was 90.2% at 20 – 26 h and the negative percent agreement was 98.9% (Table 5).

**Table 5: BBL CHROMagar MRSA II Performance vs. Traditional Culture at Third Clinical Trial Site**

CMRSA II Result	Traditional Culture		Total
	MRSA	Not MRSA	
MRSA	46	3*	49
Not MRSA	5	264	269
	51	267	318
Reference Method: Traditional Culture			
Positive Percent Agreement: 90.2% (78.6%, 96.7%)			
Negative Percent Agreement: 98.9% (96.8%, 99.8%)			

\* Two samples that were positive on **BBL CHROMagar MRSA II** and negative by Traditional Culture were confirmed as MRSA by cefoxitin disk diffusion testing.

#### Reproducibility Testing

Reproducibility testing was conducted at three clinical sites to demonstrate the ability of **BBL CHROMagar MRSA II** to provide reproducible results with known microorganisms. A blinded panel of MRSA strains and MSSA strains were provided to each site for testing. Each panel was tested in triplicate on three days at each site. For all sites, the results for this study showed  $\geq 95\%$  reproducible results within each site and across all sites for the entire panel.

#### Challenge Testing

Testing of twenty (20) challenge strains of *S. aureus* was conducted at three clinical sites using an MRSA suspension of  $10^6$  to  $10^7$  CFU/mL. Ten  $\mu$ L of this suspension was then inoculated onto **BBL CHROMagar MRSA II**. The panel included 14 MRSA (heterogeneous and homogeneous samples), and 6 MSSA. At each clinical trial site, sensitivity was 100% for the 14 MRSA strains and specificity was 100% for the 6 MSSA strains.

#### Internal Performance Evaluation

##### Recovery Rate

**BBL CHROMagar MRSA II** was evaluated to determine the recovery rate (limit of detection (LOD)) for recovery of methicillin-resistant *S. aureus*. Seven test strains, representing five heterogeneous and two homogeneous MRSA were evaluated for recovery on **BBL CHROMagar MRSA II**.<sup>15</sup> Non-selective Columbia Agar with 5% Sheep Blood plates were used to determine the organism concentration expressed in colony forming units (CFU) for each dilution.

Analytical studies including incubation time, analytical reactivity or sensitivity, interfering substances and reproducibility were all performed using an MRSA suspension of  $1 \times 10^5$  CFU/mL. Ten  $\mu$ L of this suspension was then inoculated onto **BBL CHROMagar MRSA II**.

##### Interference Study

Commonly used transport devices, nasal spray and whole blood were evaluated for potential interference and inhibition of MRSA on **BBL CHROMagar MRSA II**.

Nasal sprays containing fluticasone propionate, azelastine hydrochloride and oxymetazoline hydrochloride as well as OTC throat drops containing menthol demonstrated antibacterial activity. No other substances or transport devices interfered with recovery of MRSA on **BBL CHROMagar MRSA II**.<sup>16</sup>

##### Cross Reactivity

Internal testing of other *Staphylococcus* and non-*Staphylococcus* organisms was conducted in order to determine the potential cross reactivity of these organisms with **BBL CHROMagar MRSA II**. Two hundred and eighty-five non-MRSA organisms were tested, including the following genera: *Acinetobacter*, *Aerococcus*, *Aeromonas*, *Bacillus*, *Bacteroides*, *Burkholderia*,

*Campylobacter, Candida, Chryseobacterium, Citrobacter, Clostridium, Corynebacterium, Cryptococcus, Edwardsiella, Enterobacter, Enterococcus, Escherichia, Eubacterium, Hafnia, Klebsiella, Kocuria, Kytococcus, Lactobacillus, Micrococcus, Moraxella, Morganella, Neisseria, Oerskovia, Planococcus, Plesiomonas, Prevotella, Proteus, Providencia, Pseudomonas, Rhodococcus, Rothia, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus* and *Vibrio*.

In the Cross Reactivity study, strains of *Chryseobacterium meningosepticum, Corynebacterium jeikeium, Enterococcus faecalis* (VRE), *Rhodococcus equi, Bacillus cereus, Staphylococcus simulans, S. epidermidis*, and methicillin-susceptible *Staphylococcus aureus* produced mauve-colored colonies.

Overall analytical specificity of isolate testing was 97.3% at 24 h.

#### Sensitivity (Analytical Reactivity)

Internal testing of methicillin-resistant *Staphylococcus aureus* was conducted in order to determine sensitivity of the organism with **BBL CHROMagar MRSA II**. Two hundred and ninety-two MRSA including USA 100, and USA 300 isolates were evaluated on **BBL CHROMagar MRSA II** using a suspension of 10<sup>5</sup> CFU/mL. Ten µL of this suspension was then inoculated onto **BBL CHROMagar MRSA II**. Overall analytical sensitivity of isolate testing was 92.7% at 24 h.

Twenty-seven of the two hundred and ninety-two MRSA isolates which demonstrated non-mauve or no growth results during the analytical reactivity testing were further evaluated on **BBL CHROMagar MRSA II** using a suspension of 10<sup>6</sup> CFU/mL. Ten µL of this suspension was then inoculated on to **BBL CHROMagar MRSA II**. Twenty-five of the twenty-seven isolates evaluated produced mauve colonies on **BBL CHROMagar MRSA II** at 24 h at this concentration.

#### XIV AVAILABILITY

Cat. No.	Description
215228	<b>BBL™ CHROMagar™ MRSA II</b> , Pkg. of 20 plates
215229	<b>BBL™ CHROMagar™ MRSA II</b> , Ctn. of 100 plates

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