BBL™ CHROMagar™ MRSAII*

INTENDED USE

BBL™ CHROMagar™ MRSAII (CMRSAII) is a selective and differential medium for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens. The test can be performed on respiratory (e.g., nares, throat and sputum), lower gastrointestinal (GI) (e.g., rectal and stool), skin (e.g., groin/axilla and perineum/perianal), and wound specimens, and positive blood culture bottles containing gram-positive cocci.

SUMMARY AND EXPLANATION

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).[1] Selection of these organisms has been greatest in the healthcare setting; however, MRSA has also become more prevalent in the community.[2]

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 MRSAII is a selective and differential medium, which incorporates cefoxitin for the detection of MRSA from respiratory (e.g., nares, throat and sputum), lower GI (e.g., rectal and stool), skin (e.g., groin/axilla and perineum/perianal), and wound specimens, and positive blood culture bottles containing gram-positive cocci.

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

PRINCIPLES OF THE PROCEDURE

Microbiological Method

BBL CHROMagar MRSAII 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[3] 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 blue to blue/green colored colonies or if no chromogenic substrates are utilized, the colonies appear as white or colorless.

***European, U.S. & Canadian Patents Pending**

REAGENTS

BBL CHROMagar MRSAII

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

pH: 6.9 +/- 0.2 at 25°C

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

Warnings and Precautions

IVD For professional use only.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus may be present in clinical specimens. "Standard Precautions"[4-7] 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.[8]

Storage Instructions: On receipt, store plates in their original wrapping and box at 2-8°C until time of inoculation. Minimize exposure (< 4h) of **BBL CHROMagar MRSAII** 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.

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

SPECIMEN COLLECTION AND HANDLING 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.[9, 10]

PROCEDURE

Materials Provided:
BBL CHROMagar MRSAII (90 mm **Stacker™** plates) 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: The medium can be used for respiratory (e.g., nares, throat, and sputum), lower GI (e.g., rectal and stool), skin (e.g., groin/axilla and perineum/perianal), and wound specimens, and positive blood culture bottles containing gram-positive cocci.

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.

Respiratory, lower GI, skin and wound specimens*:* As soon as possible after receipt in the laboratory, inoculate a **BBL CHROMagar MRSAII** plate and streak for isolation. Incubate plates aerobically at 35-37°C for 18-28 h in an inverted position. If no mauve colonies are recovered, reincubate for a total of 36-52 h.

Positive blood culture bottles containing gram-positive cocci*:* As soon as the blood culture bottle is designated as positive and the Gram stain confirms the presence of gram-positive cocci, remove an aliquot, inoculate a **BBL CHROMagar MRSAII** plate and streak for isolation. Incubate plates aerobically at 35-37°C for 18-28 h in an inverted position. Incubation beyond 18-28 h is not required.

Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation as light may destroy the chromogens. Exposure to light is permissible after colony color develops.

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 control organisms that produce known, desired reactions. *S. aureus* ATCC™ 29213 may be tested directly or tested at a concentration of 10^4 - 10^5 CFU/plate to confirm the presence of cefoxitin.[11] *S. aureus* ATCC 43300 may be tested directly or tested at a concentration of 10^3 -10^4 CFU/plate to determine the growth capacity of the medium and the performance of the chromogenic reaction.[11]

Test Strain	Expected Results
Staphylococcus aureus ATCC™ 43300 (MRSA)	Growth of mauve colonies
Staphylococcus aureus ATCC™ 29213 (MSSA)	No growth

Quality control requirements must be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures. The user may refer to CLSI guidance for appropriate quality control practices.

RESULTS

Read plates against a white background. Colonies of MRSA will appear mauve on the **BBL CHROMagar MRSAII** medium. Other organisms (non-MRSA) will be inhibited or produce blue to blue/green, white or colorless colonies. Refer to Tables 1 and 2 for interpretation of results.

Table 1 Interpretation of results for respiratory, lower GI, skin and wound specimens

18-28 h Incubation	Interpretation/Recommended Action
Mauve colonies morphologically resembling staphylococci*	MRSA detected
No mauve colonies	Reincubate for a total of 36-52 h

36-52 h Incubation	Recommended Action	Interpretation
Mauve colonies*	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	N/A	No MRSA detected

*Staphylococci typically produce moderately sized smooth mauve colonies on **BBL CHROMagar MRSAII** medium. Mauve colonies which are very small to pinpoint are most often gram-positive rods, usually *corynebacteria*. A confirmatory test such as coagulase or *Staphylococcus* latex agglutination should be performed at 36 -52 h and may be performed directly from the **BBL CHROMagar MRSAII** plate.

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

18-28 h Incubation	Interpretation/Recommended Action
Mauve colonies morphologically resembling staphylococci*	MRSA detected
No mauve colonies	No MRSA detected

*Staphylococci typically produce moderately sized smooth mauve colonies on **BBL CHROMagar MRSAII** medium. Mauve colonies which are very small to pinpoint are most often gram-positive rods, usually *corynebacteria*. If incubated beyond 18-28 h, a confirmatory test such as coagulase or *Staphylococcus* latex agglutination should be performed and may be performed directly from the **BBL CHROMagar MRSAII** plate.

LIMITATIONS OF THE PROCEDURE

Minimize exposure of **BBL CHROMagar MRSAII** to light (<4 h) 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 MRSAII** has been optimized for incubation at 35-37°C for 18-28 h. Lower incubation temperatures (<35° C) and/or shorter incubation times (<18 h) may reduce the sensitivity of **BBL CHROMagar MRSAII**.

Incubation time beyond 36 - 52 h is not recommended.

At 36 -52 hours incubation, occasional strains of *Chryseobacterium meningosepticum,* coagulase-negative *Staphylococcus* spp*., Corynebacterium* spp*., Enterococcus* spp*., Lactobacillus* spp*.,* methicillin-sensitive *Staphylococcus aureus, Morganella morganii, Proteus* spp*., Rhodococcus equi, Serratia marcescens* and yeast may produce mauve colonies requiring a coagulase test or Staphylococcus latex agglutination for confirmation of MRSA. This may also occur at a much lower rate at 18-28 h.

*mec*A-negative *S. aureus* may grow if the oxacillin or cefoxitin MICs are at or near the resistant breakpoint.

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

Rare strains of MRSA have demonstrated sensitivity to the **BBL CHROMagar MRSAII** 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.

A heavy bacterial load and/or some specimen components may result in 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. This phenomenon should not be interpreted as positive.

Before using **BBL CHROMagar MRSAII** for the first time, training on the typical colony appearance of MRSA with defined strains; e.g., the strains mentioned under **User Quality Control**, is recommended.

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 *S. aureus* related hospitalizations have increased 62% and the estimated number of methicillin - resistant *S. aureus* hospitalizations more than doubled from 1999 through 2005.[12] 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%. Dramatic increases in the incidence of soft tissue and skin infections were found, suggesting community - associated MRSA is spreading in hospitals.[12, 13]

PERFORMANCE CHARACTERISTICS

BBL CHROMagar MRSAII is used for the qualitative direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from respiratory (e.g., nares, throat, and sputum), lower GI (e.g., rectal and stool), skin (e.g., groin/axilla and perineum/perianal), and wound specimens, and positive blood culture bottles containing gram-positive cocci.

External Performance Evaluation

BBL CHROMagar MRSAII was evaluated at four diverse clinical laboratories with remnant, prospective respiratory (e.g., nares, throat, and sputum), lower GI (e.g., rectal and stool), skin (e.g., groin/axilla and perineum/perianal) and wound specimens, and positive blood culture bottles containing gram-positive cocci. Specimens were evaluated by comparing the recovery of MRSA on traditional culture media (e.g., Tryptic Soy Agar with 5% Sheep Blood, Columbia Agar with 5% Sheep Blood, or CNA (colistin nalidixic acid agar), depending upon specimen types) and **BBL CHROMagar MRSAII** plates. *S. aureus* recovered on the traditional culture media were tested by the cefoxitin disk diffusion test method. Cefoxitin disk diffusion test results followed CLSI interpretive criteria for the determination of methicillin resistance (R) and methicillin susceptibility (S), (R ≤ 21mm and S ≥ 22 mm).[3, 14] **BBL CHROMagar MRSAII** was interpreted as positive for MRSA at 18-28 h based on detection of mauve colonies or at 36 - 52 h based on detection of mauve colonies with confirmation as *S. aureus.*

The overall prevalence of MRSA from **BBL CHROMagar MRSAII** was 15% (778/5051), or about 65.6% (778/1186) of all *S. aureus.* For the traditional culture plate (e.g., Tryptic Soy Agar with 5% Sheep Blood, Columbia Agar with 5% Sheep Blood, and CNA) the MRSA recovery rate was 89.8% (621/778), while for **BBL CHROMagar MRSAII**, the MRSA recovery rate was 95.6% (744/778).

Table 3 MRSA Recovery: **BBL CHROMagar MRSAII** vs. Traditional Culture

		MRSA Recovery	
Specimen Category	**Read Time[1]**	**Traditional Culture**	**CMRSAII**
Respiratory	**24 h**	79.8% (182/228)	85.5% (195/228)
	48 h	76.8% (182/237)	92.4% (219/237)
Lower GI	**24 h**	86.9% (93/107)	87.9% (94/107)
	48 h	77.5% (93/120)	98.3% (118/120)
Skin	**24 h**	68.6% (118/172)	88.4% (152/172)
	48 h	66.3% (118/178)	96.1% (171/178)
Wound	**24 h**	90.6% (115/127)	92.1% (117/127)
	48 h	88.5% (115/130)	94.6% (123/130)
Blood Culture[2]	**24 h**	100% (113/113)	100% (113/113)
Combined[3]	**24 h**	**83.1% (621/747)**	**89.8% (671/747)**
	48 h	**79.8% (621/778)**	**95.6% (744/778)**

[1] 24 h represents a read range of 18-28 h with no confirmatory testing required and 48 h read range is 36-52 h with confirmatory testing.

[2] Positive blood culture containing gram-positive cocci

[3] Includes all specimen types (respiratory, lower GI, skin, wound and blood culture)

Table 4: **BBL CHROMagar MRSAII** Performance vs. Traditional Culture and Cefoxitin Disk by Specimen Type

		Cefoxitin Disk	
Specimen Category	**Read Time[1]**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Respiratory	24 h	**85.5%** (195/228) (80.3%,89.8%)	99.8% (1216/1218) (99.4%,100%)
	48 h	**92.4%** (219/237) (88.3%,95.4%)	99.8% (1207/1209) (99.4%,100%)
Lower GI	24 h	**87.9%** (94/107) (80.1%,93.4%)	100% (587/587) (99.4%,100%)
	48 h	**98.3%** (118/120) (94.1%,99.8%)	100% (574/574) (99.4%,100%)
Skin	24 h	**88.4%** (152/172) (82.6%,92.8%)	100% (1103/1103) (99.7%,100%)
	48 h	**96.1%** (171/178) (92.1%,98.4%)	100% (1097/1097) (99.7%,100%)
Wound	24 h	**92.1%** (117/127) (86%,96.2%)	100% (821/821) (99.6%,100%)
	48 h	**94.6%** (123/130) (89.2%,97.8%)	100% (818/818) (99.6%,100%)
Blood Culture[2]	24 h	**100%** (113/113) (96.8%,100%)	100% (575/575) (99.4%,100%)
Combined[3]	24 h	**89.8% (671/747) (87.4%,91.9%)**	**100% (4302/4304) (99.8%,100%)**
	48 h	**95.6% (744/778) (93.9%,97%)**	**100% (4271/4273) (99.8%,100%)**

[1] 24 h represents a read range of 18-28 h with no confirmatory testing required and 48 h read range is 36-52 h with confirmatory testing.

[2] Positive blood culture containing gram positive cocci

[3] Includes all specimen types (respiratory, lower GI, skin, wound and blood culture)

Respiratory specimens:

A total of 1446 respiratory specimens were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** was higher at 92.4% (219/237), compared to a recovery of 76.8% (182/237) on traditional culture plates at 48 h. At the 18-28 h reading, two false positives were observed on **BBL CHROMagar MRSAII**, for a specificity of 99.8% (1216/1218). Using colony color at the18-28 h reading for **BBL CHROMagar MRSAII**, and confirming all mauve colonies with a confirmatory test at the 36-52 h reading, the overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for respiratory specimens was 98.6% (1426/1446).

Lower GI specimens:

A total of 694 lower GI specimens were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** was higher at 98.3% (118/120) compared to a recovery of 77.5% (93/120) on traditional culture plates at 48 h. There were no false positive specimens observed on **BBL CHROMagar MRSAII**. Using colony color at the 18-28 h reading for **BBL CHROMagar MRSAII** and confirming all mauve colonies with a confirmatory test at the 36-52 h reading, the overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for lower GI specimens was 99.7% (692/694).

Skin specimens:

A total of 1275 skin specimens were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** was higher at 96.1% (171/178) compared to a recovery of 66.3% (118/178) on traditional culture plates at 48 h. There were no false positive specimens observed on **BBL CHROMagar MRSAII**. Using colony color at the 18-28 h reading for **BBL CHROMagar MRSAII**, and confirming all mauve colonies with confirmatory testing at the 36-52 h reading, the overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for skin specimens was 99.5% (1268/1275).

Wound specimens:

A total of 948 wound specimens were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** was higher at 94.6% (123/130) compared to a recovery of 88.5% (115/130) on traditional culture plates at 48 h. There were no false positives observed on **BBL CHROMagar MRSAII**. Using colony color at the 18-28 h reading for **BBL CHROMagar MRSAII**, and confirming all mauve colonies with confirmatory testing at the 36-52 h reading, the overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for wound specimens was 99.3% (941/948).

Positive blood culture bottles containing gram-positive cocci:

A total of 688 positive blood culture bottles containing gram-positive cocci were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** and traditional culture plates was equivalent at 100% (113/113) at 18-28 h. There were no false positives observed on **BBL CHROMagar MRSAII**. Using colony color at the 18-28 h reading for **BBL CHROMagar MRSAII**, the overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for positive blood culture bottles was 100% (688/688).

Combined specimen types:

A combined overall total of 5051 specimens were evaluated comparing the recovery of MRSA on traditional culture plates to **BBL CHROMagar MRSAII** plates. Overall recovery of MRSA on **BBL CHROMagar MRSAII** 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 MRSAII**, for a specificity of 99.9% (4271/4273). Using colony color at the 18-28 h reading for **BBL CHROMagar MRSAII**, and confirming all mauve colonies with confirmatory testing at the 36-52 h reading, the combined overall agreement of **BBL CHROMagar MRSAII** compared to the cefoxitin disk diffusion test for all specimen types was 99.3% (5015/5051).

Challenge Testing

Testing of twenty (20) challenge strains of *S. aureus* was conducted at three clinical sites. The panel included 14 MRSA and 6 MSSA. Individual sites and combined site agreements were 100%.

Internal Performance Evaluation

Limits of Detection (LOD)

BBL CHROMagar MRSAII was evaluated to determine the limit of detection (LOD) of methicillin-resistant *S. aureus* recovery. Four test strains; representing two heterogeneous and two homogeneous MRSA were evaluated for recovery on **BBL CHROMagar MRSAII**[15]. 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. The LOD for CMRSAII ranged from 4-116 CFU at 24 h and 4-24 CFU at 48 h[16].

Interference Study

A total of 30 substances including commonly used medicinal substances, transport devices, enrichment broth, and blood culture media were evaluated for potential interference and inhibition of MRSA on **BBL CHROMagar MRSA II**. Some mouthwash, throat drops, acetylsalicylic acid, personal lubricants and ibuprofen may reduce recovery of MRSA. At a concentration of 10%, a nasal spray containing phenylephrine hydrochloride demonstrated antibacterial activity. No other substances, devices or media tested interfered with recovery of MRSA on **BBL CHROMagar MRSA II**.[16]

AVAILABILITY

Cat. No.	Description
REF 257434	**BBL™ CHROMagar™ MRSAII** Ready-to-use Plated Media, cpu 20
REF 257435	**BBL™ CHROMagar™ MRSAII** Ready-to-use Plated Media, cpu 120

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