I INTRODUCTION
BD 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
1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation. For Enterococcus faecalis ATCC® 29212 and Staphylococcus aureus ATCC 25923 and 29213, dilute cultures to yield $10^4$–$10^5$ CFU/plate. For Staphylococcus aureus ATCC 33591 and 43300 dilute cultures to yield $10^3$–$10^4$ CFU/plate.
   b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
      NOTE: Minimize exposure to light before and during incubation.
   c. Include BD Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 and 42–48 h for recovery, colony size, and color.
3. Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
<th>ATCC</th>
<th>Recovery</th>
<th>Colony Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212</td>
<td>Inhibition (partial to complete)</td>
<td>No growth or non-mauve colonies</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923</td>
<td>Inhibition (partial to complete)</td>
<td>No growth or non-mauve colonies</td>
</tr>
<tr>
<td>*Staphylococcus aureus</td>
<td>29213</td>
<td>Inhibition (partial to complete)</td>
<td>No growth or non-mauve colonies</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>33591</td>
<td>Growth</td>
<td>Mauve</td>
</tr>
<tr>
<td>*Staphylococcus aureus</td>
<td>43300</td>
<td>Growth</td>
<td>Mauve</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control. Direct inoculation may be used for User Quality Control.

NOTE: Before using BD 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
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 7.0 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE
BD 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.

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

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.

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

BD BBL CHROMagar MRSA II is a modified version of the existing formulation of BD 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
BD 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 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
BD 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” 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.

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. 9,10

IX PROCEDURE
Material Provided: BD 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 BD 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 BD 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 BD BBL CHROMagar MRSA II medium. Refer to Table 1 for interpretation of results.

Table 1: Interpretation of results for anterior nares specimens

<table>
<thead>
<tr>
<th>20–26 h Incubation</th>
<th>Interpretation/Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mauve colonies morphologically resembling staphylococci</td>
<td>Positive - MRSA detected</td>
</tr>
<tr>
<td>Non-mauve colonies detected*</td>
<td>Negative - No MRSA detected</td>
</tr>
<tr>
<td>No growth</td>
<td>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.</td>
</tr>
</tbody>
</table>

*Certain MRSA may produce non-mauve colonies on BD 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 BD 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 BD 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 BD BBL CHROMagar MRSA II.
- MRSA concentrations of lower than 10^6 CFU/mL may yield false negative results on BD 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.
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%. Data from the NNIS (National Nosocomial Infections Surveillance System) indicate that in the intensive care patient setting, the proportion of MRSA infections has increased to 59.5–64.4%. 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

BD 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 BD Trypticase Soy Agar with 5% Sheep Blood (TSA II) plates and each site’s routine procedure for identification of *S. aureus* (Traditional Culture) to BD 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. BD BBL CHROMagar MRSA II was interpreted as positive for MRSA at 20–26 h based on detection of mauve colonies.

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

<table>
<thead>
<tr>
<th>Cefoxitin Disk</th>
<th>CMRSA II Result</th>
<th>MRSA</th>
<th>Not MRSA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>149</td>
<td>1</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Not MRSA</td>
<td>13</td>
<td>1,024</td>
<td>1,037</td>
<td></td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>1,025</td>
<td>1,187</td>
<td></td>
</tr>
</tbody>
</table>

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 BD 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: BD BBL CHROMagar MRSA II Performance vs. Cefoxitin Disk

<table>
<thead>
<tr>
<th>Positive Percent Agreement (95% CI)</th>
<th>Negative Percent Agreement (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>92% (149/162) (86.7%, 95.7%)</td>
<td>99.9% (1,024/1,025) (99.5%, 100%)</td>
</tr>
</tbody>
</table>

With combined data from two clinical trial sites, the positive percent agreement of BD 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: BD BBL CHROMagar MRSA II Performance vs. Traditional Culture at Two Clinical Trial Sites

<table>
<thead>
<tr>
<th>Traditional Culture</th>
<th>CMRSA II Result</th>
<th>MRSA</th>
<th>Not MRSA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>92</td>
<td>9*</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Not MRSA</td>
<td>8</td>
<td>760</td>
<td>768</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>769</td>
<td>869</td>
<td>100</td>
</tr>
</tbody>
</table>

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 BD 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 BD 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: BD BBL CHROMagar MRSA II Performance vs. Traditional Culture at Third Clinical Trial Site

<table>
<thead>
<tr>
<th>Traditional Culture</th>
<th>CMRSA II Result</th>
<th>MRSA</th>
<th>Not MRSA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>46</td>
<td>3*</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Not MRSA</td>
<td>5</td>
<td>264</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>267</td>
<td>318</td>
<td>51</td>
</tr>
</tbody>
</table>

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 BD 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 BD 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 ≥ 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 µL of this suspension was then inoculated onto BD 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
BD 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 BD BBL CHROMagar MRSA II. 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 1x10^5 CFU/mL. Ten µL of this suspension was then inoculated onto BD 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 BD 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 BD BBL CHROMagar MRSA II.
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 **BD 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*, *Kyrtococcus*, *Lactobacillus*, *Micrococcus*, *Moraxella*, *Morganella*, *Neisseria*, *Oerskiovia*, *Planococcus*, *Plesiomonas*, *Prevotella*, *Proteus*, *Providencia*, *Pseudomonas*, *Rhodococcus*, *Rothia*, *Salmoneilla*, *Seratia*, *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 **BD BBL CHROMagar** MRSA II. Two hundred and ninety-two MRSA including USA 100, and USA 300 isolates were evaluated on **BD BBL CHROMagar** MRSA II using a suspension of 10^5 CFU/mL. Ten µL of this suspension was then inoculated onto **BD 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 **BD BBL CHROMagar** MRSA II using a suspension of 10^6 CFU/mL. Ten µL of this suspension was then inoculated on to **BD BBL CHROMagar** MRSA II. Twenty-five of the twenty-seven isolates evaluated produced mauve colonies on **BD BBL CHROMagar** MRSA II at 24 h at this concentration.

**XIV AVAILABILITY**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>215228</td>
<td><strong>BD BBL™ CHROMagar™</strong> MRSA II, Pkg. of 20 plates</td>
</tr>
<tr>
<td>215229</td>
<td><strong>BD BBL™ CHROMagar™</strong> MRSA II, Ctn. of 100 plates</td>
</tr>
</tbody>
</table>

**XV REFERENCES**


Becton, Dickinson and Company
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Sparks, MD 21152 USA

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