BD BBL™ CHROMagar™ MRSA II

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QUALITY CONTROL PROCEDURES

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 10^3–10^5 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 h for recovery, colony size, and color.
3. Expected Results

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

*Recommended organism strain for User Quality Control. Direct inoculation may be used for User Quality Control.[1]

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 6.9 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 30 ± 1 °C for 60 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 by trained personnel. 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).[2] Selection of these organisms has been greatest in the healthcare setting; however, MRSA has also become more prevalent in the community.[3]

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.[2]

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[4] 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 in Singapore.

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± 2 °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:

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

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 **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 ± 2 °C for 20–26 h. Lower incubation temperatures (< 33 °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.

- 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 $1x10^9$ 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 **BD BBL CHROMagar** MRSA II, therefore the performance of **BD 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 **BD 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 **BD 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.[11]
- 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 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 AVAILABILITY

Cat. No.	Description
252353	**BD BBL™ CHROMagar™** MRSA II, Pkg. of 20 plates
252354	**BD BBL™ CHROMagar™** MRSA II, Ctn. of 100 plates

XIII REFERENCES

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