



QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar™ MRSA, supplemented with chromogens and inhibitory agents, is used for the qualitative direct detection of nasal colonization by methicillin-resistant *Staphylococcus aureus*.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak the plates for isolation. For *Staphylococcus aureus* ATCC™ 43300 and 33591, use an 18–24 h broth culture diluted to yield 10^3 – 10^4 CFU/plate. Use an 18–24 h broth culture for all other organisms diluted to yield 10^4 – 10^5 CFU/plate.
 - b. Incubate plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.

NOTE: Minimize exposure to light before and during incubation.

- c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h and 42–48 h for recovery, colony size and pigmentation.
 3. Expected results

Organisms	ATCC™	Recovery	Colony Color
<i>Staphylococcus aureus</i>	29213	Inhibition (partial to complete)	N/A
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	N/A
* <i>Staphylococcus aureus</i>	43300	Growth	Mauve
<i>Staphylococcus aureus</i>	33591	Growth	Mauve
<i>Enterococcus faecalis</i>	29212	Growth	Blue

*Recommended organism strain for User Quality Control.

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 6.8 ± 0.2 .
4. Note the firmness of the plates during the inoculation procedure.
5. Incubate uninoculated representative plates at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ MRSA is a selective and differential 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 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.

V SUMMARY AND EXPLANATION

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 Selection of these organisms has been greatest in the healthcare setting; however, MRSA have 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 MRSA is a selective and differential medium, which incorporates cefoxitin, for the detection of MRSA from anterior nares specimens.

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

VI PRINCIPLES OF THE PROCEDURE

BBL CHROMagar MRSA 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 cefoxitin³ and produce 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.

VII REAGENTS

BBL CHROMagar MRSA

Approximate Formula Per Liter Purified Water

Chromopeptone	40.0 g	Inhibitory Agents	0.07 g
Sodium Chloride	25.0 g	Cefoxitin	6.0 mg
Chromogen Mix.....	0.5 g	Agar	14.0 g

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"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.

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

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 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.^{8,9}

IX PROCEDURE

Material Provided: BBL CHROMagar MRSA

Materials Required But Not Provided: Ancillary culture media, coagulase test reagents, quality control organisms 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** plate and streak for isolation. Incubate plates aerobically at 35–37°C for 24 ± 4 h in an inverted position. If no mauve colonies are recovered, reincubate for an additional 24 ± 4 h. Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation (>4 h) as light may result in reduced recovery and/or coloration of isolates. 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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Read plates against a white background. Colonies of MRSA will appear 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
Mauve colonies morphologically resembling staphylococci*		MRSA detected, report MRSA nasal colonization
No mauve colonies		No result available, reincubate 24 additional hours
48 h Incubation	Recommended Action	Interpretation
Mauve colonies	Perform coagulase testing	If coagulase positive – MRSA detected, report MRSA nasal colonization If coagulase negative – report no MRSA detected
No mauve colonies	N/A	Report no MRSA detected

*Staphylococci typically produce moderately sized smooth 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.

XI LIMITATIONS OF THE PROCEDURE

Minimize exposure (<4 h) of **BBL CHROMagar MRSA** 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 cardboard box for the entire storage period.

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., *Corynebacterium* 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, 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 coagulase test may be performed at 24 h on mauve-colored colonies to increase specificity.

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. The test is not intended to identify patients with staphylococcal infection. Results should not be

used to guide or monitor treatment for MRSA infections. This device 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.

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

Incubation in 5% CO₂ 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 inhibitory effect on organism growth that is unrelated to medium performance.

Rare strains of MRSA have demonstrated sensitivity to the **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.

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 between 25 and 30%.¹¹ 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 2003.¹²

In the external clinical evaluation of **CHROMagar** MRSA, 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. aureus*. 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).

XIII PERFORMANCE CHARACTERISTICS

Clinical Studies

CHROMagar MRSA was evaluated at four geographically diverse hospitals with fresh prospective surveillance specimens of the anterior nares. A total of 1,974 surveillance nares 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. epidermidis*, 2 *S. haemolyticus*, and 2 *Corynebacterium*). 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 2–5:

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

Table 2

		TSA II Result			
		Growth of <i>S. aureus</i>			
		Oxacillin MIC Reference Result			
CHROMagar MRSA Result	MRSA Identification	MRSA	MSSA	No growth of <i>S. aureus</i>	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 **CHROMagar** MRSA: 15 were confirmed as MRSA by positive PBP 2' 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 **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	
% Agreement of MRSA (95% CI)	% Agreement of MSSA (95% CI)
94.9% (111/117) (89.2%, 98.1%)	96.6% (201/208) (93.2%, 98.6%)

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

Table 4

		TSA II Result			Total
		Growth of <i>S. aureus</i>		No growth of <i>S. aureus</i>	
		Oxacillin Screen Agar Reference Result			
CHROMagar MRSA Result	MRSA Identification	MRSA	MSSA		
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 **CHROMagar MRSA**: 15 were confirmed as MRSA by positive PBP 2' 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 **CHROMagar MRSA: 45 were confirmed as coagulase-negative staphylococci; and 23 were gram-positive rods and other organisms.

Table 5

CHROMagar MRSA vs. Oxacillin Screen Agar	
% Agreement of MRSA (95% CI)	% Agreement of MSSA (95% CI)
94.8% (110/116) (89.1%, 98.1%)	96.7% (202/209) (93.2%, 98.6%)

These studies also compared **CHROMagar MRSA** to other test methods for identifying MRSA and MSSA: 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). 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 Disk Diffusion		CHROMagar MRSA vs. PBP 2' Latex Agglutination		CHROMagar MRSA vs. PCR (<i>mecA</i>)	
% 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%)

Challenge Testing

Testing of twenty (20) challenge strains of *S. aureus* was conducted at three of the 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 all 100%, and site and overall specificities were 100%.

Expression of Resistance

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 1,000,000 cells expressing resistance,¹³ making detection by conventional antimicrobial susceptibility tests difficult. Fifteen test strains, representing 10 heterogeneous and 5 homogeneous MRSA, were evaluated for recovery and colony counts on **CHROMagar MRSA** compared to a nonselective medium, TSA II with 5% sheep blood. Both **CHROMagar MRSA** and TSA II recovered all 15 strains. **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 **CHROMagar MRSA** is able to detect both homogeneous and heterogeneous strains.¹⁴

Interference Study

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 **CHROMagar MRSA** medium. At a 10% concentration, a nasal spray containing phenylephrine hydrochloride demonstrated antibacterial activity on **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 **CHROMagar MRSA** medium.¹⁴

XIV AVAILABILITY

Cat. No.	Description
215084	BBL™ CHROMagar™ MRSA , Pkg. of 20 plates
215181	BBL™ CHROMagar™ MRSA , Ctn. of 100 plates

XV REFERENCES

1. Muto, C. A., J. A. Jernigan, B. E. Ostrowsky, H. M. Richet, W. R. Jarvis, J. M. Boyce, B. M. Farr. 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. *Infect. Control and Hospital Epidemiol.* May 362-386.
2. Bannerman, T. L. 2003. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenenbaum (eds.), 8th ed., Manual of clinical microbiology. ASM, Washington DC.
3. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; Eighteenth Informational Supplement, M100-S18. CLSI, Wayne, PA.
4. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed., CLSI, Wayne, PA.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17: 53-80.
6. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, DC.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
8. Ramsay-Shea, Y. 1992. Specimen collection and transport. *In* Isenberg, H.D. (ed.), Clinical microbiology procedures handbook. ASM, Washington DC.
9. Miller, J.M., H.T. Holmes, K. Krishner. 2003. General principles of specimen collection and handling. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenenbaum (eds.), 8th ed., Manual of clinical microbiology. ASM, Washington DC.
10. Clinical and Laboratory Standards Institute. 2004. Approved Guideline M22-A3. Quality assurance for commercially prepared microbiological culture media, 3rd ed., CLSI, Wayne, PA.
11. MRSA – Methicillin Resistant *Staphylococcus aureus*: Fact Sheet. CDC website, <http://www.cdc.gov/ncidod/hip/Aresist/mrsafaq.htm>.
12. Proportion of *S. aureus* Nosocomial Infections Resistance to Oxacillin (MRSA) Among Intensive Care Unit Patients, 1989 – 2003 (graph). CDC website, http://www.cdc.gov/ncidod/hip/AREISIT/ICU_MRSA.pdf.
13. Tomasz A., Nachman S., Leah H. 1991. Stable classes of phenotypic expression in methicillin resistant clinical isolates of staphylococci. *Antimicro. Agents Chemother.* 35:124-129.
14. Data on file, BD Diagnostics.

U.S. Patent Pending

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