



Spectra™ MRSA

INTENDED USE

Remel Spectra™ MRSA is a selective and differential chromogenic medium recommended for use in the qualitative detection of nasal colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA in healthcare settings. The test is performed with anterior nares swab specimens from patients and healthcare workers to screen for MRSA colonization. Spectra™ MRSA is not intended to diagnose MRSA infection or to guide or monitor treatment for infections.

Spectra™ MRSA is also intended for use in the qualitative detection of MRSA from positive blood cultures demonstrating gram-positive cocci on Gram stain. Spectra™ MRSA is indicated for use in conjunction with other laboratory tests and clinical data available to the clinician as an aid in the detection of MRSA from patient positive blood cultures. Spectra™ MRSA is not intended to monitor treatment for MRSA infections, or provide results of susceptibility to methicillin. All positive blood bottles should be subcultured for further microbiological/susceptibility testing.

SUMMARY AND EXPLANATION

Soon after the introduction of methicillin in 1959, methicillin-resistant *S. aureus* (MRSA) was reported in the United Kingdom.¹ Though rare in the United States until the mid-1970s, MRSA currently accounts for more than sixty percent of *S. aureus* hospital-acquired infections.² In June 2007, the Association for Professionals in Infection Control and Epidemiology (APIC) released results from the first nationwide study on the burden of MRSA on U.S. healthcare. The data showed that 46 out of every 1,000 patients (4.6%) were infected or colonized with MRSA; a rate up to eleven times greater than previous estimates. Experts at the Centers for Disease Control and Prevention reported that MRSA cause more than 94,000 life-threatening (invasive) infections and 19,000 deaths in the U.S. Klevens et al. confirmed this finding and estimated 18,900 MRSA hospital-onset bacteremias occur annually.³

The Healthcare Infection Control Practices Advisory Committee (HICPAC) issued recommendations for the management of Multidrug-Resistant Organisms (MDROs), including MRSA, in healthcare settings in 2006.⁴ Implementation of active surveillance cultures (ASC) to identify colonized patients and Contact Precautions are strongly recommended as control measures to reduce MDRO transmission.

In the U.S., treatment of MRSA-infected hospitalized patients is associated with increased length of stay and complications requiring costly critical care stays, resulting in an annual cost estimated at \$3.2-4.2 billion.⁵ Rapid, accurate, and cost-effective screening tests for MRSA colonization are needed in order to reduce the economic burden of this pathogen. The use of selective culture media utilizing specific chromogens has been described as reliable and fast for screening for MRSA in comparison to traditional culture methods.

PRINCIPLE

Remel Spectra™ MRSA is an opaque medium, which uses a novel chromogen that yields a denim blue color as a result of phosphatase activity. This enzyme is present in all MRSA. To allow the medium to differentiate MRSA accurately, it contains a combination of antibacterial compounds designed to inhibit the growth of a wide variety of competitor organisms. Also included are compounds that encourage the production of MRSA pathogenicity marker, ensuring expression of the phosphatase enzyme and so providing enhanced sensitivity and specificity.

REAGENTS (CLASSICAL FORMULA)*

Peptone Mix	25.0 g
Salt Mix	25.0 g
Kaolin	13.0 g
Chromogenic Mix	2.0 g
Selective Agents	4.0 ml
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 7.3 +/- 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁶

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Positive blood cultures from the following blood culture systems BacT/ALERT®, VersaTREK®, and BACTEC™, (see Tables 2 and 3 in the Performance Characteristics section for blood culture bottle types), (7) Microscope slide, Gram Stain reagents.

PROCEDURE

Allow plates to equilibrate to room temperature prior to inoculation.

Nasal Swabs

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. Incubate plates in ambient air for 24 hours at 35-37°C.
3. Observe colony characteristics, morphology, and color reactions.

Positive Blood Cultures

1. Gram stain positive blood cultures for evidence of gram-positive cocci characteristic of *Staphylococcus* species.
2. Inoculate Spectra™ MRSA from the positive blood culture bottle following manufacturer recommendations. (Traditional non-selective culture media should also be inoculated.)
3. Incubate plates in ambient air for 24 hours at 35-37°C.
4. Observe colony characteristics, morphology, and color reactions.

INTERPRETATION OF THE TEST

After 24 hours incubation, MRSA will appear as small to medium denim blue colonies against a white background. The colonies are typically smaller than on non-selective media. Other organisms (non-MRSA) will exhibit marked inhibition or produce white colonies. If after 24 hours incubation no denim blue colonies are observed, the specimen is considered negative and plates should be discarded.

24 hours incubation	Interpretation Nasal Colonization	Interpretation Positive Blood Culture Gram-positive cocci
Small to medium denim blue colonies	Positive – MRSA colonization	Positive – MRSA infection or bacteremia
No denim blue colonies	Negative – No MRSA colonization	Negative – No MRSA detected

Rare strains of *Staphylococcus cohnii* and methicillin-resistant *Staphylococcus epidermidis* may grow and produce very dark, navy-blue and pale-blue colonies respectively. The intensity of the color reaction enables differentiation from MRSA. If in doubt, confirm with a latex agglutination test directly from the Spectra™ MRSA plate. Infrequently, *Bacillus cereus* may grow and produce denim blue colonies that are medium size, irregular, and flat with an undulate margin, which are morphologically distinct from MRSA.

QUALITY CONTROL

All lot numbers of Spectra™ MRSA have been tested using the following quality control organisms and have been found to be acceptable. Quality control testing must be performed in accordance with applicable local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Staphylococcus aureus</i> ATCC® 43300	Ambient, 24 h @ 35-37°C	Denim blue
<i>Staphylococcus aureus</i> ATCC® 29213	Ambient, 24 h @ 35-37°C	No growth

PROCEDURE FOR TESTING QUALITY CONTROL ORGANISMS^a

1. Use a pure, 18-24 hour culture of each quality control organism.
2. Prepare a suspension of each organism in sterile, nonbacteriostatic saline (0.85% w/v NaCl) equal in density to a 0.5 McFarland standard (1x10⁷ to 1x10⁸ cfu/ml).
3. Dilute the suspension 1:10 in sterile broth or nonbacteriostatic saline.
4. Inoculate Spectra™ MRSA with 10 µl (0.01 ml) of the diluted suspension using a calibrated loop or pipette and streak for isolation.
5. Incubate and read results at 24 hours.

^aRefer to Clinical and Laboratory Standards Institute (CLSI) M22.⁷

LIMITATIONS

1. Organisms with atypical enzyme patterns may give anomalous results.
2. Incubation in CO₂ may reduce recovery and potentially result in a false negative reaction.
3. The growth requirements of certain MRSA can lead to their partial or complete inhibition in culture. A false-negative MRSA result may occur when testing borderline oxacillin-resistant strains of *S. aureus* (BORSAs) or modified *S. aureus* (MODSAs). These strains have oxacillin MICs of 4-8 µg/ml.
4. Surveillance testing determines the colonization status at a given time and can vary depending on patient treatment, patient status (actively shedding), or exposure to high-risk environments. Monitoring of colonization status should be performed in accordance with hospital policies and procedures.

5. Neutralization of antimicrobial activity by dilution in the blood culture media varies depending on the dosage level, susceptibility of the microorganism, and timing of specimen collection. The use of supplementary additives should be considered in appropriate situations. Users should be cognizant of manufacturer information for Blood Culture systems regarding the recovery of microorganisms.
6. The performance of Spectra™ MRSA has not been established for blood culture bottle types other than those shown in Table 2.
7. The performance of Spectra™ MRSA when testing pediatric blood cultures is unknown because insufficient pediatric samples were evaluated and none were positive for MRSA.
8. In preclinical recovery studies conducted with low inoculum, a fewer number of colonies than expected were observed for the heteroresistant MRSA (ATCC® 43300). The observation was most notable for VersaTrek® Redox 1 and Redox 2 blood culture bottles. The reasons for this observation are not known.

EXPECTED VALUES

Forty-six of every 1,000 U.S. hospital inpatients are colonized or infected with MRSA, according to APIC findings in a 2006 survey of more than 1,200 hospitals. Roughly 77% of these MRSA patients were admitted to the hospital already infected or colonized. An estimated 18,900 MRSA hospital-onset bacteremias occur annually in the U.S. The overall prevalence rate of MRSA colonization in this study was 14.2%. For blood cultures exhibiting gram-positive cocci characteristic of *Staphylococcus* species on Gram stain, 28% were positive for MRSA.

PERFORMANCE CHARACTERISTICS

Clinical Accuracy:

Nasal Colonization

The performance of Spectra™ MRSA was evaluated at four geographically diverse regions of the United States. A total of seven hundred sixty-seven (767) prospective anterior nares surveillance specimens were tested. Results from the Spectra™ MRSA at 24 hours incubation were compared to results obtained from traditional culture on Tryptic Soy Agar with 5% Sheep Blood (Blood Agar) after 48 hours incubation. During the course of this study, one swab was used to inoculate both plates, with the Blood Agar plate being inoculated first for all specimens. The overall recovery of MRSA on Spectra™ MRSA at 24 hours was 95.4% (104/109) compared to recovery of 96.3% (105/109) on Blood Agar at 48 hours.

Suspect isolates of *S. aureus* were identified from Spectra™ MRSA using a latex agglutination test or a biochemical identification system. Susceptibility testing was performed using an antibiotic gradient method for oxacillin and the Oxoid PBP2' test for the detection of the penicillin-binding protein 2a. The overall agreement for detection of MRSA and non-MRSA by denim blue colonies isolated on Spectra™ MRSA at 24 hours compared to identification and susceptibility testing as described was 99.1% (760/767). The positive and negative predictive values for Spectra™ MRSA compared to the Oxoid PBP2' test were 98.1% and 99.2% respectively.

Table 1: Nasal Colonization Performance

	MRSA	Non-MRSA
Spectra™ MRSA vs. traditional culture*	95.2% (100/105) (95% CI = 89.2–98.4%)	99.1% (656/662) (95% CI = 98.0–99.7%)
Spectra™ MRSA vs. PBP2'	95.4% (104/109) (95% CI = 89.6–98.5%)	99.7% (656/658) (95% CI = 98.9–100%)
Spectra™ MRSA vs. Oxacillin MIC	95.4% (104/109) (95% CI = 89.6–98.5%)	99.7% (656/658) (95% CI = 98.9–100%)

*4 MRSA positive specimens were negative by traditional culture.

Note: CI = Confidence Interval

Positive Blood Culture

The performance of Spectra™ MRSA was evaluated at three geographically diverse regions of the United States. A total of five hundred forty-seven (547) positive blood cultures exhibiting gram-positive cocci on initial microscopic examination were tested. Results from the Spectra™ MRSA at 24 hours incubation were compared to results obtained from traditional culture on Tryptic Soy Agar with 5% Sheep Blood (Blood Agar) after 48 hours incubation. The overall recovery of MRSA on Spectra™ MRSA at 24 hours was 96.2% (152/158) compared to recovery of 100% (158/158) on Blood Agar at 48 hours.

Suspect isolates of *S. aureus* were identified from Spectra™ MRSA using a latex agglutination test or a biochemical identification system. Susceptibility testing was performed using an antibiotic gradient method for oxacillin and the Oxoid PBP2' test for the detection of the penicillin-binding protein 2a. The overall agreement for detection of MRSA and non-MRSA by denim blue colonies isolated on Spectra™ MRSA at 24 hours compared to identification and susceptibility testing as described was 98.9% (541/547). The positive and negative predictive values for Spectra™ MRSA compared to the Oxoid PBP2' test were 97.4% and 99.0% respectively.

Table 2: Performance by blood culture bottle type

Blood Culture Information	Blood Culture Bottle Type	MRSA Agreement vs. PBP2' n/N [%] (95% CI)	Non-MRSA Agreement vs. PBP2' n/N [%] (95% CI)
BacT/ALERT® (vs. PBP2)	FA (FAN® Aerobic)	38/39 [97.4%] (86.5 – 99.9%)	79/79 [100%] (95.4 – 100%)
	FN (FAN® Anaerobic)	12/12 [100%] (73.5 – 100%)	100% [41/41] (91.4 – 100%)
	SYSTEM (Combined)	50/52 [96.2%] (86.8 – 99.5%)	100% [119/119] (96.9 – 100%)
VersaTREK® (vs. PBP2)	REDOX 1® (aerobic)	42/43 [97.7%] (87.7 – 99.9%)	91/93 [97.8%] (92.4 – 99.7%)
	REDOX 2® (anaerobic)	9/10 [90.0%] (55.5 – 98.9%)	7/7 [100%] (59.0 – 100%)
	SYSTEM (Combined)	53/56 [94.6%] (85.1 – 98.9%)	97/97 [100%] (96.3 – 100%)
BACTEC™ (vs. PBP2)	Plus Aerobic/F	29/30 [96.7%] (82.8 – 99.9%)	101/102 [99.0%] (94.7 – 100%)
	Lytic/10 Anaerobic/F	18/18 [100%] (81.5 – 100%)	72/73 [98.6%] (92.6 – 100%)
	SYSTEM (Combined*)	47/48 [97.9%] (88.9 – 100%)	173/175 [98.9%] (95.9 – 99.9%)

*Excludes 73 Lytic/10 Anaerobic/F or Standard/10 Aerobic/F because of the inability to separate and 8 PEDS PLUS™/F because none were positive for MRSA.

Table 3: Overall Performance of all blood culture bottle types

Blood Culture Information	Comparison to:	MRSA Agreement n/N [%] (95% CI)	Non-MRSA Agreement n/N [%] (95% CI)
All (with exclusions^{a,b})	vs. Traditional Culture	152/158 [96.2%] (91.9 – 98.6%)	389/389 [100%] (99.1 – 100%)
	vs. PBP2'	148/152 [97.4%] (93.4 – 99.3%)	391/395 [99.0%] (97.4 – 99.6%)
	vs. Oxacillin MIC	152/158 [96.2%] (91.9 – 98.6%)	389/389 [100%] (99.1 – 100%)

^aExcludes 73 Lytic/10 Anaerobic/F or Standard/10 Aerobic/F because of the inability to separate AND 8 PEDS PLUS™/F because none were positive for MRSA.

^bSix hundred twenty eight (628) positive blood cultures exhibiting gram-positive cocci on initial microscopic examination were tested. A total of 81 Spectra™ MRSA results obtained from subculture of the following blood culture bottles were excluded from the primary analysis for the reasons stated:

1. BACTEC™ Lytic/10 Anerobic/F and Standard/10 Aerobic/F because of the inability to separate the data for each bottle type
2. BACTEC™ Peds Plus because none were positive for MRSA

Challenge Testing:

Sixteen well-characterized strains of MRSA representing Pulse-Field Types USA100, USA200, USA300, USA400, USA500, USA600, USA700, USA800, USA1000, and USA1100, and four strains of MSSA were evaluated on Spectra™ MRSA and produced the expected results at 24 hours.

Interfering Substances:

Commonly used medicinal substances and transport media, as well as human blood and mucous were evaluated for potential interference of the chromogenic reaction of Spectra™ MRSA. No interference was observed.

Cross Reactivity:

Two hundred ninety-seven (297) microorganisms representing gram-negative rods, yeast, streptococci, enterococci, staphylococci and related organisms were evaluated with Spectra™ MRSA at 10⁵–10⁶ colony-forming units per ml concentration. No cross-reactivity (denim blue colonies) was observed following 24 hours incubation.

Reproducibility:

Reproducibility testing was conducted at six sites on three separate days with twenty blinded strains of *S. aureus* (MRSA, MSSA, and BORSAs). MRSA and MSSA strains produced the expected result with Spectra™ MRSA 100% of the time at 24 hours. BORSAs demonstrated variable results.

Challenge Testing:

Limit of Detection Study:

A study was conducted to determine the limit of detection of Spectra™ MRSA for the recovery of MRSA from seeded blood culture bottles and to evaluate its ability to detect MRSA in the presence of other organisms that could potentially be present in the positive blood culture. Two MRSA strains (ATCC® 33591 and ATCC® 43300) were evaluated alone or in the presence of methicillin-susceptible *S. aureus* (MSSA ATCC® 29213) and/or methicillin-resistant coagulase negative *Staphylococcus* (MR-CoNS). Simulated blood culture bottles containing MRSA (10⁴ CFUs per bottle) alone or in conjunction with MSSA and/or MR-CoNS (ranging from 10⁴ to 10⁸ CFUs per bottle) were incubated for 6 hours at 35°C prior to subculture on Spectra™ MRSA. Data from this study showed that at an initial inoculum of 10⁴ CFUs per blood culture bottle, MRSA are successfully detected on Spectra™ MRSA, when present alone or in mixed cultures. Due to the enrichment process involved in incubated blood cultures, almost all positive blood culture specimens contain very high bacterial loads resulting in CFUs that may be well above the estimated LoD of this medium.

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

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PACKAGING

Spectra™ MRSA:

REF R01821, 85 mm Plate 10/Pk
REF R01822, 85 mm Plate..... 100/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

U.S. Patent Pending

Spectra™ is a trademark of Thermo Fisher Scientific and its subsidiaries. ATCC® is a registered trademark of American Type Culture Collection. BACTEC™ is a trademark of Becton, Dickinson and Company. Bact/ALERT® is a registered trademark of bioMérieux, S.A. VersaTREK® is a registered trademark of Magellan Biosciences, Inc.

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