

MRSASelect™ Combined Nasal and Wound specimens

REF 63747

Selective and differential chromogenic culture medium for the qualitative detection of nasal colonization of methicillin resistant *Staphylococcus aureus* (MRSA) from anterior nares specimens as well as the qualitative detection of MRSA from skin and soft-tissue wound specimens.

1. INTENDED USE

MRSASelect™ is a selective and differential chromogenic medium for:

A) The qualitative detection of nasal colonization of methicillin resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test can be performed on anterior nares specimens from patients and healthcare workers to screen for MRSA colonization. **MRSASelect™** is not intended to diagnose MRSA infection nor to guide or monitor treatment of infection. Results can be interpreted after 18 to 28 hours incubation

B) The qualitative detection of methicillin resistant *Staphylococcus aureus* (MRSA) from skin and soft-tissue wound specimens. The **MRSASelect™** is indicated for use in conjunction with other laboratory tests and clinical data available to aid in the identification and diagnosis of MRSA from patients with skin and soft-tissue infections. Concomitant cultures and susceptibility testing are necessary for all skin and soft-tissue wound specimens. **MRSASelect™** is not intended to guide, or monitor treatment for MRSA infection, or provides results of susceptibility to methicillin. Results can be interpreted after 18 to 28 hours incubation.

2. SUMMARY AND EXPLANATION

Methicillin resistant *Staphylococcus aureus* is a major cause of nosocomial and life threatening infection. MRSA infections have been associated with high rates of mortality and morbidity.¹

To aid in the control and 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 an infection control program to control the spread of MRSA.¹

MRSA infections are no longer limited to hospitalized or healthcare associated patients and arise in community.² MRSA infections in community are usually manifested as skin infections.³

The Bio-Rad **MRSASelect™** is a selective and differential chromogenic culture medium for the qualitative detection of MRSA from:

- anterior nares specimens to aid in the prevention and control of MRSA in healthcare settings.
- skin and soft-tissue wound specimens, when used in conjunction with other laboratory tests and clinical data to aid in the identification and diagnosis of MRSA from patients with skin and soft-tissue infections.

3. PRINCIPLES OF THE PROCEDURE

MRSASelect™ is a selective medium for the detection and direct identification of MRSA. The selectivity of this medium is based on the presence of an antibiotic/antifungal mixture and an optimized salt concentration that inhibits the growth of yeast and the majority of Gram negative and Gram positive bacteria, with the exception of methicillin-resistant staphylococci. Identification is based on the cleavage of a

chromogenic substrate by a specific enzymatic activity of *Staphylococcus aureus*, leading to a strong pink coloration of the *Staphylococcus aureus* colonies. Plates may be read within 18-28 hours incubation.

Within 18 - 28 hours incubation:

- Methicillin-resistant *Staphylococcus aureus* produce small pink colonies on **MRSASelect™** (See Limitation c).
- Coagulase negative methicillin-resistant staphylococci do not metabolize the chromogenic substrate and appear as colorless or white colonies (possibly light/faint pink) (See Limitation e).
- Methicillin-susceptible staphylococci (MSS) are inhibited.

4. REAGENTS

MRSASelect™ (catalog # 63747) contains 20 plates per package.

Approximate media formulation (g/L)

○ Peptone	18.5
○ Silica	15.0
○ Sodium Pyruvate	1.0
○ Salt mixture	25.0
○ Chromogenic substrate	0.2
○ Agar	15.0
○ Antimicrobial and antifungal	0.1

5. WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use

Observe aseptic technique and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other potentially contaminated materials must be sterilized or disposed of in accordance with defined laboratory procedures.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus may be present in clinical samples. Universal precautions and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids.⁴

The Material Safety Data Sheet (MSDS) is available upon request or on www.bio-rad.com.

6. STORAGE INSTRUCTIONS

Store plates at 2 – 8 °C protected from light.

Prolonged exposure to light may result in reduced recovery and/or coloration of the QC organisms or patient isolates. Store the plates in original packaging until ready to use. Close plate packaging each time after any plates are removed. Plates must be used before the expiration date indicated on the label and printed on the plate.

7. PRODUCT DETERIORATION

Do not use plates if they show any evidence of contamination, drying, cracking or any other sign of deterioration.

8. SPECIMEN COLLECTION AND HANDLING

This device has been evaluated with anterior nares specimens and wound specimens. Use of transport devices approved for collection of such specimens is recommended. Follow the transport device manufacturer's recommended procedures⁵ (See Limitation o).

9. PROCEDURE

Materials Provided

- Bio-Rad **MRSASelect™** agar plates

Materials Required but not Provided

- Ancillary culture media
- QC organisms
- Other laboratory equipment as required

Test Procedure

The **MRSASelect™** agar surface should be smooth and moist. Allow the media to warm to room temperature protected from light before inoculation. Follow aseptic techniques when using the media.

If swabs are not processed immediately upon receipt, refrigerate until processed.

Inoculation on **MRSASelect™**

- Directly from patient specimen. Inoculate the sample onto the plate and streak for isolation.
- Indirect (for anterior nares specimens only): Place the swab in 0.5 mL sterile saline. Vortex for approximately 20 seconds. Inoculate immediately. Using a swab or disposable loop, transfer approximately 50 µL of the suspension onto **MRSASelect™** and streak for isolation.

Incubation

- Incubate the inoculated **MRSASelect™** in an inverted position, in ambient air for 18-28 hours at 35-37°C, protecting the plate from light.

10. RESULTS

Read the plate after 18 - 28 hours incubation. MRSA will appear as small pink colonies and non-MRSA organisms are inhibited or appear as white or colorless colonies.

18 - 28 hours incubation	Interpretation
Small Pink colonies	MRSA detected (Positive)
No Pink colonies	MRSA not detected (Negative)

After 18 - 28 hours incubation time, Methicillin resistant *Staphylococcus aureus* typically appear as small pink colonies. The size of *Staphylococcus aureus* colonies on the **MRSASelect™** is smaller than that observed on conventional media (TSA with Blood).

If white colonies are seen after 18 hours incubation, plates should be re-incubated and read after 24 hours incubation (See Limitations c and e).

Presence of pink colonies at 18-28 hours incubation represents a positive culture, and is indicative of MRSA colonization. Plates should not be incubated longer than 28 hours.

Some strains of *Staphylococcus epidermidis* may appear as small or pinpoint colonies with a very faint pink color. Re-incubate and read after 24 hours incubation. If colonies appear still as pinpoint colonies with a very faint pink color after 24 hours incubation, these should be considered negative for MRSA. (See Limitation e)

If within 18-28 hours incubation, there is a question as to the final identification of organisms isolated, pink colonies isolated on **MRSASelect™** can be further tested using Bio-

Rad Pastorex™ Staph Plus to confirm identification of *S. aureus*.

If within 18 - 28 hours incubation, no pink colonies are observed; the sample is considered negative; i.e no MRSA colonization for nasal specimens and no MRSA has been detected on **MRSASelect™** for wound specimens.

11. USER QUALITY CONTROL

Examine plates for signs of deterioration (See Section 7 above). Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions.

- Strains recommended to be tested:

S. aureus ATCC 25923

Test organisms at a concentration of 10⁴ – 10⁵ CFU/plate.

S. aureus ATCC 43300

Test organisms at a concentration of 10³ – 10⁴ CFU/plate.

Test Strain	Expected Results after 18-28 hours at 35-37°C
<i>S. aureus</i> ATCC 25923	No Growth
<i>S. aureus</i> ATCC 43300	Growth – small pink colonies

Quality control testing must be performed in accordance with local, state, and federal regulations or accreditation requirements and your laboratory's standard quality control procedures. Refer to pertinent CLSI (NCCLS) guidance documents and CLIA regulations for appropriate Quality Control Procedures.⁶

12. LIMITATIONS OF THE PROCEDURE

a) Prolonged exposure to light may result in reduced recovery and/or coloration of the QC organisms or patient isolates. Minimize exposure of **MRSASelect™** plates to light both before and during incubation. Incubation in CO₂ may result in false negative cultures. **Incubate only in ambient air incubators.**

b) The incubation time equivalence study was performed at 10⁵ CFU/ml. Lower concentrations were not tested, and the performance of **MRSASelect™** is unknown at MRSA concentrations lower than 10⁵ CFU/ml.

c) Performance of **MRSASelect™** is optimized within 18-28 hours incubation. Plates can be read any time within this timeframe. Some MRSA strains may produce white colonies at 18 hours, **MRSASelect™** plates should be re-incubated and read after 24 hours when white or faint pink colonies are observed.

d) Some strains of *Acinetobacter* may grow as large mucoid colonies on **MRSASelect™**. Colony morphology differentiates these colonies from MRSA. Other Gram-negative rods may result in pink coloration of the media, but no growth (no colonies) will be observed at 28 hours.

e) Rare strains of *Staphylococcus epidermidis* may develop a faint pink coloration. The intensity of the colony color enables differentiation from MRSA. If in doubt, confirm the identification of pink colonies by coagulase test or Pastorex™ Staph Plus (Bio-Rad).

f) *MecA*-negative *S. aureus* may grow if oxacillin or cephalosporin MICs are very close to the resistant breakpoints.

g) The performance of **MRSASelect™** for *S. aureus* oxacillin resistance mechanisms other than *mecA* has not been evaluated. Therefore, the performance of **MRSASelect™** for Borderline Oxacillin Resistant *Staphylococcus aureus* (BORSA), and modified *S. aureus* (MOD-SA) is unknown.

h) The growth requirements of certain Methicillin-resistant *Staphylococcus aureus* can lead to their partial or total inhibition in culture on **MRSASelect™**.

i) Use of the following compounds has an inhibitory effect on MRSA growth that is unrelated to medium performance: Bactine (Benzalkonium chloride 0.13%, Lidocaine hydrochloride 2.5%); Betadine (liquid) (Povidone-iodine 10%), Iodine Tincture (liquid) (Iodine 2%), Biseptine (liquid) (Chlorhexidine Gluconate 0.25%, Benzalkonium chloride 0.025%), Sodium hypochlorite (liquid), StaphAseptic (ointment) (Benzethonium Chloride 0.2%, Lidocaine HCl 2.5%), and silver chloride.

j) Use of antibiotic ointment Neosporin or Polysporin may result in decreased growth of MRSA on non-selective media as well as on **MRSASelect™**.

k) The potential impact Mupirocin may have on the performance of **MRSASelect™** has not been determined.

l) Reduced growth of MRSA (pink colonies) on **MRSASelect™** as compared to a non-selective growth media may be indicative of an oxacillin heterogeneous resistant strain. Further susceptibility testing is required to confirm the possibility of heterogeneous resistance.

m) Some MRSA strains may only grow and produce pink colonies on **MRSASelect™** at higher concentrations of $\geq 10^5$ CFU/mL

n) Performance of **MRSASelect™** has been evaluated with only non-prescription debriding agents such as 1U/g fibrinolysin and 666U/g desoxyribonuclease, Papain+urea (10%+10%); these may not be representative of all debriding agents available.

o) Performance of **MRSASelect™** has been determined at 10^5 CFU/ml with the following transport media: Carey Blair, Liquid Stuart, Amies with Charcoal and Amies without Charcoal. Performance of other transport media has not been evaluated.

p) For anterior nares specimens, 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 of colonization status should be done according to hospital policies.

q) For anterior nares specimens, use of phenylephrine hydrochloride or oxymetazoline hydro-chloride components found in some nasal sprays have an inhibitory effect on organism growth that is unrelated to medium performance.

13. EXPECTED VALUES

The prevalence of MRSA infections has increased dramatically in hospitals and, importantly, the carriage rate of MRSA is rising in the community. Recent studies suggest that 25-30% of the population is colonized with *Staphylococcus aureus*, and the prevalence of MRSA is approximately 1%.⁷ Between 15 % up to 74% of purulent skin and soft tissue infections are caused by MRSA, the most common identifiable cause of such infections.⁸ According to CDC data, the proportion of antimicrobial resistant infections has been growing. In 2004 MRSA infections accounted for 63% of the total number of *Staphylococcus* infections.⁹

In the external wound specimens clinical evaluation of **MRSASelect™**, the overall prevalence of MRSA colonization, as found by routine culture and Tryptic Soy Broth (TSB) with 6.5% NaCl was 24.2% (228/943). The prevalence detected using routine culture alone was 22.9%

(216/943) and the prevalence detected by **MRSASelect™** was 22.6% (213/943).

14. PERFORMANCE CHARACTERISTICS

A) Anterior nares specimens

Performance of **MRSASelect™** was evaluated at three geographically diverse hospitals with fresh surveillance specimens from the anterior nares. The recovery of MRSA on **MRSASelect™** was compared to routine culture, which was defined as isolation of staphylococci on Trypticase Soy Agar with 5% blood, with identification confirmed by coagulase and oxacillin susceptibility. 1772 samples were tested against routine culture. Performance of **MRSASelect™** was also compared to a commercially available chromogenic medium. 3013 samples were tested against the chromogenic medium. Product performance is summarized below:

Routine Culture media, 24 hours				
		Pos	Neg	Total
MRSA Select™	Pos	227	33	260
	Neg	10	1502	1512
24 hours	Total	237	1535	1772

% Agreement: Pos = 96%; Neg = 98%

Commercial Chromogenic media, 48 hours				
		Pos	Neg	Total
MRSA Select™	Pos	297	24	321
	Neg	18	2674	2692
24 hours	Total	315	2698	3013

% Agreement: Pos = 94%; Neg = 99%

Further testing was done to confirm extending the incubation time.

Performance of **MRSASelect™** was evaluated at two geographically diverse hospitals with fresh surveillance specimens from the anterior nares. The recovery of MRSA on **MRSASelect™** was compared to routine culture, which was defined as isolation of staphylococci on Trypticase Soy Agar with 5% blood, with identification confirmed by coagulase and oxacillin susceptibility. Plates were incubated at 35-37° C and read after 18, 20, 24 and 28 hours incubation. No differences were noted on the plates at the extended incubation time. Results were consistent after interpretation of results at 18, 20, 24, and 28 hours incubation.

MRSASelect™	Incubation time				% Agreement
	18h	20h	24h	28h	
# Positive samples	21	21	21	21	100%
# Negative samples	179	179	179	179	100%

Interference Study

Commonly used medicinal substances and commonly used transport devices were evaluated for potential interference of the chromogenic reaction of the **MRSASelect™** medium. No interference was observed.

B) Skin and soft-tissue wound Specimens

Analytical Sensitivity

To evaluate the analytical sensitivity of the **MRSASelect™**, 102 strains of MRSA, including USA100, 200, 300, 500, 600, 700, 800, and 1000 were inoculated onto **MRSASelect™** plates at concentrations of 10³ to 10⁴ CFU/mL. USA300-0114 was also tested. 97% (99/102) sensitivity was observed after 24 hours incubation.

Method Comparison

943 skin and soft-tissue wound samples were collected and tested at four clinical laboratories in the United States. Each sample was tested on **MRSASelect™**, Trypticase Soy Agar (TSA) with 5% Sheep's Blood, and Tryptic Soy Broth (TSB) with 6.5% NaCl. Samples that were positive on TSA or TSB were confirmed with Gram stain, Pastorex™ Staph Plus, and *mecA* mediated oxacillin resistance using 30 µg/mL Cefoxitin disk (R: ≤21 mm, S: ≥ 22 mm)

The following results were obtained: specificity 99.4% (95% CI: [98.5, 99.8]) and sensitivity 91.7% (95% CI: [87.3, 94.7]). The overall prevalence of MRSA in the study was 24.2% (95% CI: [21.5, 27.0]).

MRSASelect™ vs. Routine Culture and TSB

		Routine Culture & TSB		
		Pos	Neg	Total
MRSA Select ™	Pos	209	4	213
	Neg	19*	711	730
	Total	228	715	943

* For 12/19 samples – MRSA were isolated only from TSB with 6.5% NaCl and were not isolated on initial direct culture.

Specificity: 99.4%[98.5, 99.8]

Overall % agreement: 97.6% [96.3, 98.4]

Sensitivity: 91.7%[87.3, 94.7]

Reproducibility

A panel of 6 organisms, including MRSA, MSSA and *S. epidermidis*, was evaluated on **MRSASelect™**. It was performed at concentrations of 10⁶ CFU/mL for MRSA, and 10⁸ CFU/mL for non-MRSA. The panel was tested in triplicate each day for three days at three clinical sites. Overall reproducibility was 100% after 24 hours incubation when testing this panel.

Cross Reactivity Testing (Analytical Specificity)

To evaluate the analytical specificity of the **MRSASelect™** media 35 bacterial and fungal strains found in wound or skin samples were cultured and inoculated onto **MRSASelect™** plates at a concentration of ≥10⁶ CFU/mL. No cross-reactivity was observed on any strains tested. Most strains showed no growth on **MRSASelect™** with the exception of *Corynebacterium jeikeium* and *Candida tropicalis*. With both of these organisms pinpoint white colonies were observed; these are not representative of MRSA colonies, and therefore these are not cross-reactants. No variation was seen between 24 and 28 hour incubation time.

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15. ORDERING INFORMATION

Product: **MRSASelect™**

Catalog Numbers: 63747 (20 plates)

For Customer Orders and Technical Service Call: 1-800-2-BIO-RAD (1-800-224-6723)

16. REFERENCES

1. Muto C.A, J.A Jernigan, B.E Ostrowosky, H.M Richet, W.R Jarvis, J.M Boyces, BM Farr. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *S aureus* and *Enterococcus spp* Infection Control and Hospital Epidemiology. 2003
2. Community-associated MRSA in outpatients, United States, 1999-2006. E.Klein – DL.Smith and Ramanan Laxminarayan. Emerging Infectious Diseases. Vol 15, N° 12, December 2009.
3. Community associated MRSA information for clinicians. CDC website.
http://cdc.gov/ncidod/dhqp/ar_mrsa_ca_clinicians.html
4. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risk related to exposure to biological agents at work Official Journal L262, 17/10/2000;
5. Miller JM, HT Holmes, K Krisher 2003. General principles of specimen collection and handling. Clinical Microbiology procedures handbook ASM Washington.
6. Clinical and Laboratory Standards Institute-Volume 24-Number 19-M22 A3- Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition.
7. Community-Associated MRSA Information for the Public– CDC website.
http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_public.html#5
8. Moran G.J., Krishnadasan A., Gorwitz R.J., Fosheim G.E., McDougal LK., Carey R.B., and Talan D.A.; Methicillin-Resistant *S. aureus* Infections among Patients in the Emergency Department. N Engl J Med 2006;355:666-74.
9. MRSA in Healthcare Settings – CDC website.
<http://www.cdc.gov/MRSA/index.html>

Symbol



Stored plates must be protected from light.

Certificate of analysis available on
<http://www.bio-rad.com/certificate>.



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