Selective and Differential Chromogenic Medium for the Qualitative Detection of Nasal Colonization of Methicillin Resistant *Staphylococcus aureus* (MRSA)

1) Intended Use

**MRSASelect™** is a selective and differential chromogenic medium for 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 18-28 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 recommend- ed guidelines, which include an active surveillance program to identify potential reservoirs and an infection control program to control the spread of MRSA.¹ The Bio-Rad **MRSASelect™** is a selective and differential chromogenic culture medium for the qualitative detection of MRSA from anterior nares specimens.

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™**.
- Coagulase negative methicillin-resistant staphylococci do not metabolize the chromogenic substrate and appear as colorless or white colonies (possibly light pink).
- Methicillin-sensitive staphylococci (MSS) are inhibited.

4) Reagents

- **MRSASelect™** catalog # 63747 contains 20 plates/package.

<table>
<thead>
<tr>
<th>Approximate media formulation (g/L)</th>
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</thead>
<tbody>
<tr>
<td>Peptone 18.5</td>
</tr>
<tr>
<td>Silica 15.0</td>
</tr>
<tr>
<td>Sodium Pyruvate 1.0</td>
</tr>
<tr>
<td>Salt mixture 25.0</td>
</tr>
<tr>
<td>Chromogenic substrate 0.2</td>
</tr>
<tr>
<td>Agar 15.0</td>
</tr>
<tr>
<td>Antimicrobial and antifungal 0.1</td>
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</tbody>
</table>

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.² 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 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. Use of transport devices approved for collection of such specimens is recommended. Follow the transport device manufacturer’s recommended procedures.³, ⁴

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 by streaking the sample onto the plate.
  - Indirect: 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, aerobically 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.

<table>
<thead>
<tr>
<th>18-28 hours incubation</th>
<th>Interpretation/Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small pink colonies</td>
<td>MRSA colonization (Positive)</td>
</tr>
<tr>
<td>No pink colonies</td>
<td>No MRSA detected (Negative)</td>
</tr>
</tbody>
</table>
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). Some strains of *Staphylococcus epidermidis* may appear as small or pinpoint colonies with a very faint pink color. 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. If within 18-28 hours incubation no pink colonies are observed, the sample is considered negative; no MRSA colonization. If within 18-28 hours incubation there is a question as to the final identification of organisms isolated, colonies can be transferred to general growth media for further identification.

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 $10^5$ – $10^6$ CFU/plate.
- *S. aureus* ATCC 43300
  - Test organisms at a concentration of $10^2$ – $10^4$ CFU/plate.

<table>
<thead>
<tr>
<th>Test Strain</th>
<th>Expected Results after 18-28 hours at 35-37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>No Growth</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 43300</td>
<td>Growth – small pink colonies</td>
</tr>
</tbody>
</table>

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.²

12) Limitations of the Procedure

- 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 incubators.**
- Performance of MRSASelect™ is optimized for within 18-28 hours incubation.
- 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.
- 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 a coagulase test.
- meca-negative *S. aureus* may grow if oxacillin or cephalosporin MICs are very close to the resistant breakpoint.
- The growth requirements of certain Methicillin-resistant *S. aureus* can lead to their partial or total 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 of colonization status should be done according to hospital policies.

- Use of phenylephrine hydrochloride or oxymetazoline hydrochloride 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%.⁶ According to CDC data, the proportion of antimicrobial resistant infections has been growing, and in 2004 MRSA infections accounted for 63% of the total number of staphylococcal infections.⁷

14) Performance Characteristics

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:

<table>
<thead>
<tr>
<th>Routine Culture</th>
<th>% Agreement</th>
</tr>
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<tbody>
<tr>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>MRSASelect™ 24 hours</td>
<td>227</td>
</tr>
<tr>
<td>Neg</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
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</table>

<table>
<thead>
<tr>
<th>Commercial Chromogenic Media, 48 hours</th>
<th>% Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>MRSASelect™ 24 hours</td>
<td>297</td>
</tr>
<tr>
<td>Neg</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>315</td>
</tr>
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</table>

Further testing was done to confirm extending the incubation time.

Performance of MRSASelect™ was evaluated at two geographically diverse hospitals with fresh anterior nares, surveillance specimens. 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 at 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.

<table>
<thead>
<tr>
<th>MRSASelect™ Result</th>
<th>Incubation Times</th>
<th>% Agreement</th>
</tr>
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<tbody>
<tr>
<td>18 h</td>
<td>20 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Total</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
<td>21</td>
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<tr>
<td>179</td>
<td>179</td>
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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.
15) Ordering information

Product: MRSASelect™
Catalog Number: 63747 (20 plates)

For customer Orders and technical Service Call: 1-800-2-BIO-RAD (1-800-224-6723).

16) References


Symbol

Stored plates must be protected from light.

Distributor:

Bio-Rad Laboratories
Redmond, WA 98052, U.S.A.
For Customer Orders and Technical Service Call:
1-800-2-BIO-RAD (1-800-224-6723)