SASELECT™

SELECTIVE AGAR MEDIUM FOR THE ISOLATION AND DIRECT IDENTIFICATION OF STAPHYLOCOCCUS AUREUS.

1. INTENDED USE
SaSelect™ is a selective medium for the rapid isolation of Staphylococcus aureus within 18-24 hours. Detection of specific enzyme activities allows direct identification of Staphylococcus aureus and its differentiation from other staphylococci.

2. SUMMARY AND EXPLANATION
S. aureus is a well documented pathogen. It is responsible for infections ranging from superficial to systemic. Due to the prevalence of this organism and its clinical implications, detection is of utmost importance. SaSelect™ is intended for the isolation, enumeration and identification of S. aureus based on the formation of pink to orange-colored colonies. The addition of chromogenic substrates to the medium facilitates the differentiation of S. aureus from other staphylococci.

3. PRINCIPLES OF THE PROCEDURE
SaSelect™ is a selective medium composed of:
- An optimised base for rapid growth of staphylococci.
- A mixture of antifungal agents, antibiotics and a limited salt concentration to inhibit the majority of yeasts, Gram (-) and Gram (+) bacteria other than staphylococci.
- Chromogenic substrates allow direct identification of Staphylococcus aureus (pink to orange colonies) and the differentiation of other Staphylococcus species. Phosphatase activity allows direct identification of Staphylococcus aureus colonies, which appear pink to orange, at 18-24h.
- Glycosidic activity allows differentiation of several other Staphylococcus species, which appear blue.
- In the absence of the two activities previously mentioned, the colonies appear white to yellow.

4. REAGENTS
Ready to use medium
SaSelect™ Catalog no. 63748 contains 20 plates/package.

<table>
<thead>
<tr>
<th>Approximate media formulation (g/L)</th>
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<tbody>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Salt mixture</td>
</tr>
<tr>
<td>and antifungal</td>
</tr>
<tr>
<td>Chromogenic substrate</td>
</tr>
<tr>
<td>Agar</td>
</tr>
</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. The material safety data sheet (MSDS) is available upon request or on www.bio-rad.com.

6. STORAGE INSTRUCTIONS
Store plates at 2-6°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
Refer to appropriate texts or standards for details in specimen/sample collection and handling procedures.

9. PROCEDURE
MATERIAL
Material provided: SaSelect™ agar medium
Materials required but not provided:
- Incubator, 35-37°C
- Laboratory supplies required for this procedure
Optional Materials not provided:
- Supplementary culture media
- Quality control organisms
Inoculation
The SaSelect™ 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. As soon as possible after receipt of the specimen in the laboratory, inoculate onto SaSelect™ plate and streak for isolation. Refer to current recommendations for storage of biological specimens.
It is also possible to inoculate SaSelect™ directly from a 0.5 Mc Farland saline suspension of a colony or from a positive broth culture. Ensure adequate separation of individual colonies using usual inoculation methods to ensure typical morphology.
Incubation
Incubate the inoculated SaSelect™ in an inverted position, aerobically for 18 to 24 hours at 35-37°C, protecting the plate from light.

RESULTS
Reading and Interpretation
Direct identification:
- Pink to orange colonies: Staphylococcus aureus.
- Differentiation of others staphylococci:
  - White to faint pink small colonies: Staphylococcus epidermidis probable.
  - Blue to turquoise colonies: Staphylococcus saprophyticus, Staphylococcus simulans, Staphylococcus cohnii, Staphylococcus xylosus possible.
  - Purple grey colonies: Staphylococcus intermedius probable.
- Others staphylococci are white to yellow: Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri, Staphylococcus caprae, Staphylococcus lugdunensis, Staphylococcus sciuri.

Note: If incubation is longer than 24 hours, it is recommended to confirm identification of pink/orange colonies with a supplementary test such as free coagulase or rabbit plasma, a DNase test or Pastorex™ Staph Plus (# 56356, # 56353). Colonies with coloration other than pink to orange must be identified, if necessary, by conventional methods. Interpretation of the test results should be made.

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taking into consideration the patient’s history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed.

10. PERFORMANCE CHARACTERISTICS
A prospective clinical study has been conducted on 322 samples: surface pus, deep pus, puncture fluids, blood cultures, catheter, expectoration, nasal swabs, throat swabs, ENT suppurations, urine and stool cultures. SaSelect™ and Mannitol Salt Agar (MSA/Chapman) media were inoculated in parallel and incubated at 35-37°C. A total of 152 strains of Staphylococcus aureus were isolated using the two techniques. At 18/24h, SaSelect™ detected 149 confirmed strains of Staphylococcus aureus. This study demonstrates the following:

<table>
<thead>
<tr>
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<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>SaSelect™ 24h</td>
<td>98.0% (149/152)</td>
<td>99.9 %</td>
</tr>
<tr>
<td>MSA 24h + coagulase test</td>
<td>90.8% (138/152)</td>
<td>99.4 %</td>
</tr>
<tr>
<td>MSA 48h + coagulase test</td>
<td>94.7% (144/152)</td>
<td>99.4 %</td>
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</table>

The sensitivity for coagulase negative staphylococci on SaSelect™ is improved by more than 11% compared to MSA medium.

11. USER QUALITY CONTROL
The appearance of the ready-to-use medium should be opalescent agar. 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:

<table>
<thead>
<tr>
<th>Strains</th>
<th>Result of culture at 35-37°C for 18-24h</th>
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<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Pink colonies</td>
</tr>
<tr>
<td>ATCC 25923</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Pink colonies</td>
</tr>
<tr>
<td>ATCC 43300</td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Inhibition</td>
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<tr>
<td>ATCC 25933</td>
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Test organisms at a concentration of 10⁵ – 10⁶ CFU/plate. 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.⁵

12. LIMITATIONS OF THE PROCEDURE
In an inoculum with a high Staphylococcus aureus load, the color deposit can appear more intense (orange) than for isolated colonies (pink). Some strains of Staphylococcus epidermidis and Staphylococcus schleiferi can give a pale pink color after 18/24h. The size and color intensity of the colonies make it possible to distinguish them from Staphylococcus aureus. If there is doubt, or after more than 24h incubation, it is advisable to perform a confirmatory test: Pastorex™ Staph Plus (# 56356, # 56353). The majority of microorganisms, other than staphylococci, are inhibited. However, corynebacteria, Gram-negative rod, and yeasts can sometimes grow on SaSelect™ agar. An inoculum with a high load of enterococci and streptococci, may produce a light growth of small white colonies. Nevertheless, the size, color and morphology of the colonies allow them to be distinguished from Staphylococcus aureus.

The intrinsic demands of certain staphylococci can lead to their partial or total inhibition in culture. For some samples, specifically stool culture, the color of the colony can be diminished or hidden. Dilution of the sample and streaking inoculation produces well-isolated colonies which will clearly show the enzymatic activity.

13. ORDERING INFORMATION
Product: SaSelect™
Catalog Numbers: 63748 (20 plates)
For Customer Orders and Technical Service Call: 1-800-2-BIO-RAD (1-800-224-6723)

14. REFERENCES

Symbol
Stored plates must be protected from light.