

OXACILLIN RESISTANCE SCREENING AGAR

- For in vitro use only -

Catalogue No. PO90

Our Oxacillin Resistance Screening Agar (ORSA) is used for the screening and isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical samples.

ORSA contains peptone and yeast extract, two excellent sources of carbon, nitrogen, and other vital nutrients necessary for growth. ORSA is based on traditional mannitol salt agar although the salt concentration has been reduced to allow MRSA to flourish, while still suppressing the mixed flora found in clinical samples. Mannitol is a fermentable carbohydrate utilized by coagulase-positive staphylococci including MRSA. Sugar fermentation results in acid production and the color indicator, aniline blue, gives colonies an intense blue coloration. ORSA contains three antimicrobials, oxacillin, polymyxin B, and lithium chloride. Oxacillin is used since it is the standard antibiotic used to test for MRSA and is relatively more stable than other penicillins. The ORSA oxacillin concentration (2µg/mL) is low compared to a standard MRSA screen plate (6µg/mL); this reduced level allows for quicker growth of MRSA colonies while still inhibiting MSSA. The addition of polymyxin B and lithium chloride serves to inhibit other bacteria able to grow at the reduced salt level such as Proteus species.

Formula per Litre of Medium

| 11.8 g |
|--------|
| 9.0 g |
| 10.0 g |
| 55.0 g |
| 5.0 g |
| 0.2 g |
| |

| Agar | 12.5 g |
|-------------|-----------|
| Polymyxin B | 50 000 IŬ |
| Oxacillin | 2 mg |

 $pH 7.2 \pm 0.2$

Recommended Procedure

- 1. Allow medium to reach room temperature.
- 2. Take the specimen swab and roll the swab over a small area near the edge of the plate. Then using a sterile loop, perform a fourquadrant streak, starting where the swab was inoculated, to obtain well isolated colonies.
- 3. Incubate aerobically at 35°C.
- 4. Examine after 24 hours for typical MRSA colonies. Re-incubate plates an additional 24 hours before discarding.

Interpretation of Results

MRSA colonies appear as intense blue colonies on the agar surface usually with a blue halo. Other bacteria are usually inhibited but those able to grow on the medium are typically colorless. Most MRSA show growth after 24 hours of incubation, although all culture plates must be incubated for 48 hours before a final interpretation is made. If MRSAtype colonies are observed, a coagulase test should be performed to confirm that the colony is coagulase-positive.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

• Some coagulase-negative staphylococci and enterococci may form blue colonies on this medium

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

| Organism | Expected Results |
|--|---------------------------|
| Staphylococcus aureus ATCC 43300 (MRSA) | Growth (Blue colonies) |
| Staphylococcus aureus ATCC 25923 | Inhibition |
| <i>Escherichia coli</i> ATCC 25922 | Inhibition |

Storage and Shelf Life

Our Oxacillin Resistance Screening Agar (ORSA) should be stored away from direct light at 4° C to 8° C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of two weeks from the date of manufacture.

References

- 1. Chapman GH. The significance of sodium chloride in studies of staphylococci. J Bacteriol 1945; 50:201.
- Mir N, Sanchez M, Baquero F, Lopez B, Calderon C, Canton R. Soft salt-mannitol agar-cloxacillin test: a highly specific bedside screening test for detection of colonization with methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1998; 36:986-9.
- 3. Oxoid Insert. Information Sheet No: 405 ORSAB. Oxoid, 2000.

- Simor AE, Goodfellow J, Louie L, Louie M. Evaluation of a new medium, oxacillin resistance screening agar base, for the detection of methicillin-resistant *Staphylococcus aureus* from Clinical Specimens. J Clin Microbiol 2001; 3422.
- Levi K, Bailey C, Bennett A, Marsh P, Cardy DLN, Towner KJ. Evaluation of an isothermal signal amplification method for rapid detection of methicillin-resistant *Staphylococcus aureus* from patientscreening swabs. J Clin Microbiol 2003; 41:3187-91.

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