INSTRUCTIONS FOR USE

OXACILLIN-SALT SCREEN AGAR

Ready-to-use plates

Oxacillin-Salt Screen Agar: growth of MRSA strain

1 - INTENDED USE

In vitro diagnostic. For the detection of *mec*A-mediated resistance to oxacillin of *Staphylococcus aureus* isolates.

2 - COMPOSITION -TYPICAL FORMULA *

Beet extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Sodium chloride	40.0 g
Oxacillin	0.006 g
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important etiological agent of hospital and community acquired infections. Strains that are oxacillin and methicillin resistant, historically termed methicillin-resistant *S. aureus* (MRSA), are resistant to all ß-lactam agents, including cephalosporins and carbapenems, although they may be susceptible to the newest class of MRSA-active cephalosporins (e.g. ceftaroline).[1] MRSA is resistant to all β-lactams because of the presence of *mec*A, a non-native gene encoding for a novel penicillin-binding protein (PBP2a) with a significantly lower affinity for β-lactam antibiotics. This resistance allows cell-wall biosynthesis, the target of β-lactams, to continue even in the presence of typically inhibitory concentrations of antibiotic. Non *mec*A-mediated resistance (production of PBP protein by *mec*C gene, inactivation of the drug by increased production of β-lactamase, production of modified intrinsic PBPs with altered affinity for the drug) occurs only rarely in *S.aureus*.[2] A distinctive feature of methicillin/oxacillin resistance is its heterogeneous nature, with the level of resistance varying according to the culture conditions and the β-lactam antibiotics being used. All cells in a culture may carry the genetic information (*mec*A) for resistance, but only a small number may express the resistance *in vitro* (heteroresistance).[1,2]

In addition to broth microdilution testing, the Clinical and Laboratory Standards Institute (CLSI), recommends a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplemented with 4% NaCl or the cefoxitin disk diffusion test, as alternative methods of testing for MRSA.[3] Oxacillin-Salt Screen Agar consists of Mueller Hinton Agar supplemented with sodium chloride for improving the growth of MRSA populations. Oxacillin is included as antistaphylococcal β-lactamase stable penicillin; its use is preferred because it is most resistant to degradation in storage and because it is more likely to detect heteroresistant staphylococcal strains. Oxacillin-Salt Screen Agar is used for the detection of *mec*A-mediated resistance to oxacillin of *S.aureus* and the results applied to other β-lactams agents, ie, penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), and carbapenems.[3]

4 - PHYSICAL CHARACTERISTICS

Prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Oxacillin-Salt Screen Agar	Ready-to-use plates	549510	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator, laboratory equipment as required, sterile loops, swabs, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Oxacillin-Salt Screen Agar is inoculated with pure cultures of clinical isolates presumptively identified as a *S.aureus*. It is not intended for the microbial isolation from clinical specimens.

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

- Prepare the inoculum suspension by selecting colonies from overnight growth on a non selective agar plate.
- Transfer the colonies to Trypic Soy Broth or saline solution to produce a suspension to obtain 0.5 McFarland turbidity.
- Using a 1-µL loop that was dipped in the suspension, spot an area 10 to 15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
- Incubate for a full 24 hours at 33-35°C in aerobic conditions (MRSA may not be detected with incubation at temperature above 35°C)

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it

9- READING AND INTERPRETATION

Examine the incubated plates carefully with transmitted light.

- Resistant strain (MRSA): presence of more than 1 colony or light film of growth.
- Susceptible strain: absence of colonies or presence of only1 colony.

MRS are resistant to all β-lactam agents with the exception of ceftaroline; other β-lactam agents should be reported as resistant or should not be reported.[3]

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.aureus	ATCC	29213	33-35°C / 24H / A	Susceptible: absence of growth
S.aureus	ATCC	43300	33-35°C / 24H / A	Resistant: presence of growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use plates of Oxacillin-Salt Screen Agar is tested for performance characteristics with one oxacillin susceptible strain (*S.aureus* ATCC 29213) and one oxacillin resistant strain (*S.aureus* ATCC 43300).
After incubation at 33-35°C for 24 hours in ambient air, *S.aureus* ATCC 43300 shows growth while *S.aureus* ATCC 29213 is completely inhibited.

12 - LIMITATIONS OF THE METHOD

- When performed properly, the oxacillin agar screening method will detect most *mec*A-positive *S.aureus* strains. Occasionally a heteroresistant *mec*A-positive strain is not detected, in part due to a low frequency of resistance expression.[4,5]
- The medium may not detect borderline-resistant strains with non-*mec*A-mediated resistance.[2]
- Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C.[1]
- Occasionally, *S.aureus* isolates with borderline resistant MICs may fail to grow within 24 hours. It is recommended confirming any ambiguous results demonstrated on the screening plate, by a standard MIC test.
- Oxacillin-Salt Screen Agar cannot be used for detecting oxacillin resistance in coagulase negative staphylococci.[6]
- Together with Oxacillin-Salt Screen Agar, inoculate a blood agar plate to evaluate the viability and purity of the culture.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Centers for Disease Control. Methicillin-resistant Staphylococcus aureus (MRSA): Laboratory Testing. https://www.cdc.gov/mrsa/lab/index.html. Page last reviewed: February 6, 2019.
2. Limbago BM, Swenson JM. Special phenotypic methods for detecting antimicrobial resistance. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 30th ed. CLSI supplement M100-S30. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
4. Cavassini M, Wenger A, et al. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination Kit, for rapid detection of Methicillin Resistance in Staphylococcus aureus. J Clin Microbiol 1999; 37:1591
5. Resende CA, Figueiredo AM. Discrimination of methicillin-resistant Staphylococcus aureus from borderline-resistant and susceptible isolates by different methods. J Med Microbiol 1997; 46:145
6. Tenover FC, Jones RN, Swenson JM, Zimmer B, McAllister S, Jorgensen JH. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J Clin Microbiol 1999; 37:4051

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/10
Revision 2	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it