remel

MUELLER HINTON AGAR w/ 4% NaCl and 6 µg/ml OXACILLIN

INTENDED USE

Remel Mueller Hinton Agar w/ 4% NaCl and 6 µg/ml Oxacillin is a solid medium recommended for use in qualitative procedures to screen *Staphylococcus aureus* for resistance to penicillinase-resistant penicillins (e.g., methicillin, nafcillin, and oxacillin).

SUMMARY AND EXPLANATION

In the 1950s, an increase in the number of β-lactamase-producing strains of *S. aureus* gave rise to the need for a penicillin that was resistant to staphylococcal β-lactamase. Methicillin, a penicillinase-resistant penicillin (PRP), was introduced in 1960 as a therapeutic agent active against these organisms. Soon after its release, methicillin-resistant *S. aureus* (MRSA) strains were reported in Great Britain. MRSA soon became a major nosocomial pathogen in Great Britain and Europe, but remained extremely rare in the United States until the mid-1970s. Today, MRSA continues to be one of the leading causes of nosocomial infections worldwide.[1-3]

MRSA strains are referred to as heteroresistant because two subpopulations coexist within a culture. The resistant population usually grows much more slowly than the susceptible subpopulation leading to detection problems with traditional *in vitro* susceptibility test methods. Successful detection depends largely on promoting the growth of the resistant subpopulation, which favors lower temperatures, longer incubation, and the presence of salt in the media.

PRINCIPLE

Mueller Hinton Agar contains beef extract and acid hydrolysate of casein which supply amino acids, nitrogenous substances, vitamins, and minerals necessary for growth. Agar is the solidifying agent. Starch is a protective colloid against toxic materials present in the medium. Sodium chloride has been added for better growth of methicillin-resistant *S. aureus*. Of the antistaphylococcal, β-lactamase-stable penicillins, oxacillin can be tested and results applied to the other penicillin-stable penicillins (i.e., cloxacillin and dicloxacillin). Oxacillin is preferred because it is more resistant to degradation in storage, and because it is more likely to detect heteroresistant staphylococcal strains.[4-6]

REAGENTS (CLASSICAL FORMULA)*

Sodium Chloride	40.0 g
Acid Digest of Casein	17.5 g
Beef Extract	2.0 g
Starch	1.5 g
Oxacillin	6.0 mg
Agar	17.0 g
Demineralized Water	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[7]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) 0.5 McFarland standard (REF R20410) or equivalent photometric device, (7) Micropipettor, calibrated loop.

PROCEDURE

1. Implement appropriate procedures to verify presumptive identification of the test isolate as *S. aureus*.
2. Allow plate to equilibrate to room temperature before use. The agar surface should not have excess moisture prior to inoculation.
3. Prepare a direct suspension of the test isolate in saline or broth from an 18-24 hour culture on nonselective media.
4. Select isolated colonies of the same morphological type and transfer to the saline or broth.
5. Adjust the turbidity of the suspension visually to a 0.5 McFarland standard or equivalent, or use a photometric device.
6. Using a 1 µl loop, spread the inoculum over a 10 to 15 mm area of the agar surface. Alternatively, immerse a sterile swab into the suspension and rotate it against the side of the tube above the fluid level to remove excess inoculum. Use the expressed swab to spot a 10 to 15 mm area or streak an entire quadrant of the plate.
7. After the inoculum has dried, incubate the plate in ambient air at 33-35°C (incubation at temperatures above 35°C may not detect methicillin-resistant staphylococci) for a full 24 hours.
8. Following incubation, examine plate carefully with transmitted light for > 1 colony or a light film of growth.

INTERPRETATION OF THE TEST

Resistant (MRSA) -	Growth > 1 colony
Susceptible (**not** MRSA) -	No growth or 1 colony

Note: For oxacillin-resistant *S. aureus*, cephems and other β-lactams such as amoxicillin-clavulanic acid, ampicillin-sulbactam, ticarcillin-clavulanic acid, piperacillin-tazobactam, and imipenem may appear active *in vitro* but are not effective clinically and isolates should not be reported as susceptible. This is because most cases of documented MRSA infections have responded poorly to β-lactam therapy or because convincing clinical data have not yet been presented that document clinical efficacy for those agents.[4]

QUALITY CONTROL

All lot numbers of Mueller Hinton Agar w/ 4% NaCl and 6 µg/ml Oxacillin have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[4] Controls should be included each day of testing. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Staphylococcus aureus* ATCC® 43300	Ambient, 24 h @ 33-35°C	Growth (resistant)
Staphylococcus aureus ATCC® 33591	Ambient, 24 h @ 33-35°C	Growth (resistant)
**Staphylococcus aureus* ATCC® 29213	Ambient, 24 h @ 33-35°C	No growth (susceptible)
Staphylococcus aureus ATCC® 25923	Ambient, 24 h @ 33-35°C	No growth (susceptible)

* CLSI recommended organism

LIMITATIONS

1. Clinical microbiology laboratories should regularly monitor procedures for technical human errors that may compromise the accuracy of results. Such errors include, but are not limited to: improper storage of prepared media, inoculum not properly adjusted, improper incubation temperatures, times, and/or atmospheres, transcription and reading errors interpreting results, and contamination or mutation in the control strains.[4-6]
2. This product is a screening method for use in the detection of oxacillin-resistant *S. aureus*. Data is not available for other organisms.
3. Plates should not be reused after incubation.[4]
4. A blood agar plate may be used as a growth control to monitor organism viability and purity.

BIBLIOGRAPHY

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PACKAGING

Mueller Hinton Agar w/ 4% NaCl and 6 µg/ml Oxacillin:
REF R01626, 13 x 85 mm Plate.....10/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128