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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

MRSA SCREEN PLATE

Cat. no. G47	MRSA Screen Plate, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics MRSA Screen Plate is recommended for screening *Staphylococcus aureus* isolates for resistance to methicillin, nafcillin and oxacillin.[1,7]

SUMMARY

Susceptibility testing of methicillin- or oxacillin-resistant *Staphylococcus aureus* strains is complicated by their heterogenicity. Cultures of resistant *Staphylococcus aureus* consist of various proportions of susceptible and resistant strains. In clinical specimens, resistant organisms may represent only a small percentage of the total number of organisms. These resistant strains may grow much more slowly than the susceptible population.

In 1984, Thornsberry devised a method to screen for methicillin-resistant *Staphylococcus aureus* using Mueller Hinton Agar supplemented with 4% sodium chloride and either methicillin or oxacillin. Oxacillin is used in this media due to its greater stability. Sodium chloride is incorporated in the medium to create a hypertonic environment which is more favorable for resistant organisms. Organisms that grow on this medium, in the presence of oxacillin, are considered resistant, while those that do not grow are considered susceptible. This method is recommended by CLSI (NCCLS) for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA).[1,7]

FORMULA

Ingredients per liter of deionized water:*

Beef Infusion	300.0gm
Sodium Chloride	40.0gm
Casamino Acids	17.5gm
Starch	1.5gm
Oxacillin	6.0mg
Agar	17.0gm

Final pH 7.3 +/- 0.1 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Upon receipt, store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive, protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. Isolated organisms, established isolation techniques and tests for purity are necessary before inoculating this medium. Direct inoculation will produce erroneous results. Information on specimen collection may be found in listed references.[2,3]

Method of Use:

Click For Table 2C Chart

1. Using an 18-24 hour culture from a non-selective medium, use a sweep of the culture to prepare a suspension in broth or saline equivalent to a 0.5 McFarland turbidity standard. NOTE: A sweep is needed to account for heterogenous cultures containing various populations of susceptible and resistant strains from pure culture.

2. Using a 1ul loop that was dipped in the suspension, spot an area 10-15mm in diameter. Alternatively, using a swab dipped in the suspension and expressed, spot a similar area or streak an entire quadrant.

3. Allow the inoculum to dry, then incubate in an ambient incubator at 35ºC. for 24 hours. Use and incubate each plate only once. **Do not reuse and incubate an MRSA Screen Plate.**

INTERPRETATION OF RESULTS

Examine plates at 24 hours with transmitted light for > 1 colony or a light film of confluent growth. It is important to hold the plate up to a light source, so that the light shines from behind the inoculum.

Methicillin-resistant *Staphylococcus aureus* will exhibit a light film or one or more colonies of varying size.

Determination of methicillin/oxacillin resistance in coagulase-negative staphylococci is no longer recommended on this medium.[7]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

This media is not intended for primary isolation of patient specimens. This media is used as a screen for isolates from pure culture only. Refer to listed references for more information.[1-3]

Tests to detect MRSA must be incubated for a full 24 hours at 30-35 degrees C.

Determination of methicillin/oxacillin resistance in coagulase-negative staphylococci is no longer recommended on this medium.[7]

Microbiologists should be aware that most MRSA are usually resistant to multiple antibiotics including other beta-lactams, aminoglycosides, macrolides, clindamycin and tetracycline. Staphylococci exhibiting multiple resistance may indicate methicillin resistance.

Methicillin-resistant *S. aureus* should be reported as resistant to cephams and other beta-lactams, such as amoxicillin/clavulanic acid, ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam and imipenem, regardless of the *in vitro* test results.

S. aureus exhibiting resistance to one of the penicillinase-resisitant penicillins must be reported as resistant to all beta-lactam antimicrobial agents, regardless of *in vitro* dilution test results because, in most cases of documented infections, patients have responded poorly to beta-lactam chemotherapy.[7]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, 0.5 McFarland Standard (Cat. no. ML05), and quality control organisms, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus aureus (MRSA) ATCC® 43300***	A**	24hr	35°C	Aerobic	Growth
Staphylococcus aureus ATCC® 29213***	A**	24hr	35°C	Aerobic	No growth

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** **Note:** Turbidity of inoculum is matched to a 0.5 McFarland Latex Standard (Cat. no. ML05). No further dilution is made. Using a swab or 1ul loop, the plate is spot inocultated. Results are read no later than 24 hours. Methicillin-resistant *S. aureus* (ATCC® 43300) will exhibit a faint film of growth through transmitted light or 1 or more small colonies of varying size.

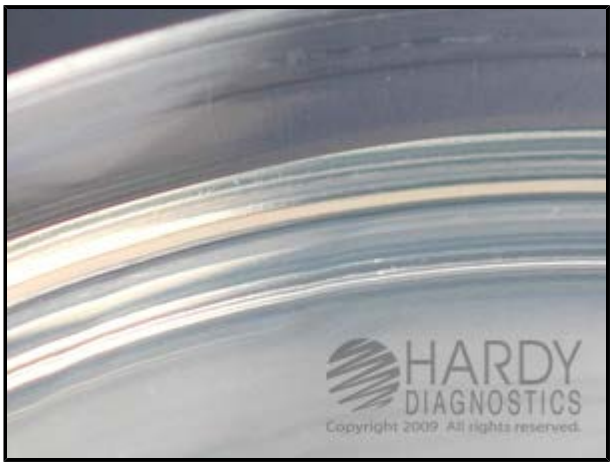
*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

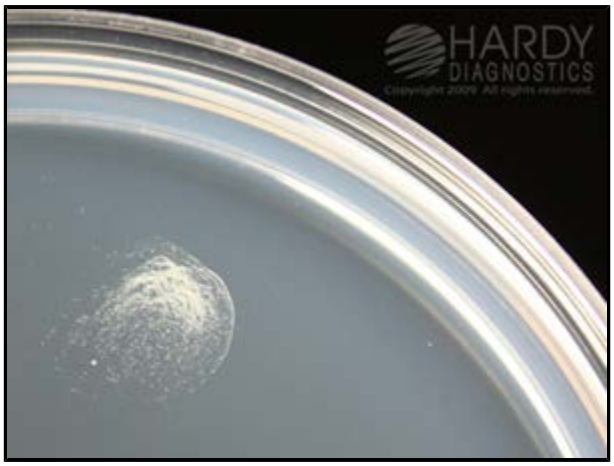
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

MRSA Screen Plate should appear clear, slightly opalescent, and white to beige in color.



Methicillin-sensitive *Staphylococcus aureus* (ATCC® 29213) growth inhibited on MRSA Screen Plate (Cat. no. G47). Incubated aerobically for 24 hours at 35ºC.



Methicillin-resistant *Staphylococcus aureus* (ATCC® 43300) colonies growing on MRSA Screen Plate (Cat. no. G47). Inoculated using the "spot" method and photographed using side-transmitted light to show growth. Incubated aerobically for 24 hours at 35ºC.

Methicillin-resistant *Staphylococcus aureus* (ATCC® 33591) colonies growing on MRSA Screen Plate (Cat. no. G47). Inoculated

REFERENCES

1. *Methods for Dilution Antimicrobial Test for Bacteria that Grow Aerobically*, M7. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology,

using the "spot" method and photographed using side-transmitted light to show growth. Incubated aerobically for 24 hours at 35ºC.

Washington, D.C.

5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

6. Thornsberry, C. and L.K. McDougal. 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (hetero-resistant) staphylococci. *J.Clin. Microbiol.*; 18:1084-1091.

7. *Performance Standards for Antimicrobial Susceptibility Testing*, M100. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]