HardyCHROM™ MRSA

Cat. no. G307	HardyCHROM™ MRSA, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

HardyCHROM™ MRSA is a selective and differential chromogenic medium recommended for the qualitative detection of nasal colonization by methicillin-resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in health care settings. The test is performed on anterior nares swabs from patients and healthcare workers to screen for MRSA colonization. HardyCHROM™ MRSA is not intended to diagnose MRSA infection nor to guide or monitor therapy for MRSA infections. Concomitant cultures are necessary to recover organisms for susceptibility testing or epidemiological typing. A negative result does not preclude MRSA nasal colonization.

SUMMARY AND PRINCIPLES

Methicillin-Resistant *Staphylococcus aureus* (MRSA) continues to be a major cause of nosocomial and life threatening infections. The prevalence of MRSA within hospital environments (Hospital Associated MRSA (HA-MRSA)) and within the community (Community Associated MRSA (CA-MRSA)) continues to increase. Infections with MRSA have been associated with high morbidity and mortality. Screening programs have been implemented in most health care settings to identify potential reservoirs so that necessary procedures can be implemented to prevent the spread of MRSA.

On HardyCHROM™ MRSA medium, methicillin-resistant *Staphylococcus aureus* strains produce pink to magenta colonies as the result of the chromogenic substrates incorporated into the medium. The addition of specific inhibitory agents allows for the growth of *mec*A mediated MRSA strains while preventing growth of methicillin sensitive *Staphylococcus aureus* (MSSA) strains. Additional selective agents have been added to increase the sensitivity and specificity of the medium by inhibiting Gram-negative organisms, yeast, and some Gram-positive cocci. Bacteria other than MRSA may utilize additional chromogenic substrates present in the medium and produce blue or green colonies. HardyCHROM™ MRSA can detect most MRSA strains within 24 hours. Negative plates should be re-incubated up to 48 hours.

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	30.0gm
Peptone	20.0gm
Chromogenic Mixture	2.0gm
Inhibitory and Selective Agents	2.5gm
Agar	15.0gm

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

Final pH 7.0 +/- 0.2 at 25 degrees C

T: 02 8212 4074 F: 02 9423 6992
www.keydiagnostics.com.au
E: infoxkeydiagnostics.com.au
PO Box 1038 Gymea NSW 2227

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C 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 applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

70 Days	G307	HardyCHROM™ MRSA

Refer to the keyword "Storage", in the Hardy Diagnostics software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdcgov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Clinical Procedure

Specimen Collection: This medium has been evaluated with anterior nares specimens. Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated into an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[2-5]

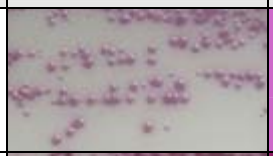
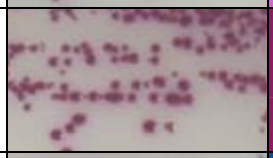
Method of Use: The plates should be warmed to room temperature. The agar surface should be dry prior to inoculating. Inoculate the specimen onto the media as soon as possible after it is received in the laboratory. If the material is being cultured from a swab, roll the swab over a small area of the agar surface and streak for isolation. Incubate plates aerobically at 35 to 37 degrees C for 24 hours. Do not incubate plates in CO_2. Observe plates for characteristic colonial morphology and color at 24 hours. If negative for MRSA, re-incubate for an additional 24 hours and read again.

If pink to magenta colonies appear at 48 hours and not at 24 hours, the organism should be subcultured to a non-selective medium for additional testing such as Gram stain (Cat. no. GK400A) and coagulase testing, (Cat. no. Z202 or ST50), before reporting as MRSA.

INTERPRETATION OF RESULTS

24 hour Incubation	Interpretation /Recommended Action
Pink to magenta colonies	Positive – MRSA detected
Purple colored film	Subculture to a non-selective medium for additional testing such as coagulase and Gram stain before reporting as MRSA.
No pink or magenta colonies detected*	Negative – No MRSA detected
No growth	Negative – A negative result does not preclude MRSA nasal colonization. If MRSA is suspected, e.g., based on patient history, an alternate method for confirming MRSA should be used.
48 hour Incubation	**Interpretation /Recommended Action**
Pink to magenta colonies	Subculture to a non-selective medium for additional testing such as coagulase and Gram stain before reporting as MRSA
No pink or magenta colonies detected*	Negative – No MRSA detected
No growth	Negative – A negative result does not preclude MRSA nasal colonization. If MRSA is suspected, e.g., based on patient history, an alternate method for confirming MRSA should be used.

* Colonies that are colorless, blue, or green should not be considered as MRSA.

Organism	Description	Photo	Color
Methicillin-resistant *Staphylococcus aureus* ATCC® 43300 24 hour incubation	pink to magenta		
Methicillin-resistant *Staphylococcus aureus* ATCC® 43300 48 hour incubation	pink to magenta		
Methicillin-resistant *Staphylococcus intermedius*	gray-blue to blue		

LIMITATIONS

1. Pink to magenta colonies seen at 48 hours of incubation should be transferred to a non-selective medium for additional testing.
2. A negative result should not be used as the sole basis for diagnosis, treatment, or management decisions. A negative result does not preclude MRSA nasal colonization.
3. Prolonged exposure to light may result in reduced recovery. Minimize exposure of HardyCHROM™ MRSA to light both before and during incubation.
4. Performance of HardyCHROM™ MRSA has been evaluated after incubation at 35-37 degrees C for 24-48 hours.
5. Do *not* incubate in a CO_2 atmosphere.

6. *mecA*-negative strains of methicillin/oxacillin resistant *S. aureus* have not been evaluated on HardyCHROM™ MRSA. It is unknown if they would grow when the oxacillin or *mecA* mediated cefoxitin MICs are at or near the resistant breakpoint.
7. Phenylephrine hydrochloride components found in some nasal sprays have been shown to have an inhibitory effect on organisms and may affect MRSA growth.
8. *Staphylococcus aureus* strains with other mechanisms of oxacillin resistance such as modified *S. aureus* (MOD-SA) strains which have altered affinity of penicillin binding proteins for oxacillin and borderline methicillin-resistant *Staphylococcus aureus* (BORSA), due to hyperproduction of betalactamases, have not been evaluated on HardyCHROM™ MRSA.
9. Use of transport media and swabs other than rayon tipped plastic shaft, nylon flocked Amies Eswabs, Stuarts gel without charcoal, Stuarts liquid, Amies liquid, Amies gel, and Amies charcoal have not been evaluated on the HardyCHROM MRSA.
10. *S. schleiferi* spp. *schleiferi* does not grow on HardyCHROM™ MRSA. *S. schleiferi* spp. *coagulans* was not evaluated on the medium and therefore, the performance is not known.
11. *S. lutrae* was not evaluated on HardyCHROM™ MRSA and therefore, the performance is not known.
12. Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ MRSA.
13. Surveillance testing determines the colonization status at a given time and could vary depending on patient treatment (e.g. decolonization regime), patient status, or exposure to high risk environments (e.g. contact with MRSA carrier or prolonged hospitalization). Monitoring colonization status should be done according to hospital policies.
14. In the event of a mixed infection, the accuracy of this device for detecting MRSA in the presence of other bacteria at a concentration of higher than 1×10^9 has not been determined and therefore is unknown.
15. Pediatric samples were not extensively analyzed during the clinical investigation; therefore, the performance of this assay with pediatric samples is unknown.
16. At 24 hours, *Corynebacterium jeikeium* produced a purple colored film. Upon further incubation, small dark purple colored colonies were seen at 48 hours. Subculture to a non-selective medium for additional testing such as coagulase and Gram stain before reporting as MRSA.
17. Reproducibility and challenge studies were performed at 10^5 to 10^6 CFU/mL concentrations. The performance of the HardyCHROM™ MRSA at recovery rate (LoD) has not been evaluated.
18. Refer to the keyword "Limitations", in the Hardy Diagnostics software program HUGO™, for more information regarding general limitations on culture media.

EXPECTED VALUES

The prevalence of HA-MRSA within hospital environments and CA-MRSA within the community continues to increase. Prevalence rates have been reported as high as 25 to 30% in the general population. In the clinical evaluation described below, the overall prevalence of MRSA was 29.5%.

PERFORMANCE CHARACTERISTICS

Performance of HardyCHROM™ MRSA was evaluated at three geographically diverse hospitals with fresh surveillance specimens collected from the anterior nares. The recovery of methicillin-resistant *S. aureus* on HardyCHROM™ MRSA was compared to routine culture, defined as isolation of staphylococci on Trypticase Soy Agar with 5% blood (TSAB), with *S. aureus* identification confirmed by latex agglutination. All recovered *S. aureus* isolates were tested for *mecA* mediated oxacillin resistance by PBP2' latex testing, cefoxitin (30µg), and oxacillin (1µg) disk diffusion. Antibiotic disk susceptibility testing followed CLSI methods and interpretive criteria[6]. Performance of HardyCHROM™ MRSA was also compared to a commercially available chromogenic medium.

A total of 443 samples were tested against routine culture. A total of 131 specimens were positive on HardyCHROM™ MRSA with concordant results obtained on TSAB and confirmed by PBP2' latex testing and cefoxitin (30µg) and oxacillin disk (1µg) diffusion testing. An additional specimen was positive on HardyCHROM™ MRSA but did not grow on TSAB. Growth of this isolate was confirmed as MRSA by PBP2' latex testing and cefoxitin (30µg) and oxacillin disk (1µg) diffusion testing. Product performance is summarized below:

Table 1: Agreement between Traditional Culture, a Commercial Chromogenic Medium and HardyCHROM™ MRSA

	MRSA	**Non-MRSA***
HardyCHROM™ MRSA vs. Traditional Culture 24 hours	93.3% (126/135) (95% CI 89.6 – 98.4%)	99.7% (307/308) (95% CI 98.9 – 100%)
HardyCHROM™ MRSA vs. Traditional Culture 48 hours	97.0% (131/135) 95% CI 89.6 – 98.5%	99.7% (307/308) 95% CI 98.9 – 100%
HardyCHROM™ MRSA vs. a Commercial Chromogenic Medium 24 hours	98.3% (118/120) 95% CI 97.5 – 99.3%	97.5% (315/323) 95% CI 89.6 – 98.4%
HardyCHROM™ MRSA vs. a Commercial Chromogenic Medium 48 hours	98.4% (122/124) 95% CI 97.5 – 99.3%	96.9% (309/319) 95% CI 89.6 – 98.4%

*****Organisms producing other types of non-*mec*A resistance were not evaluated on this medium (e.g. MOD-SA and BORSA)

Table 2: Comparison between HardyCHROM™ MRSA and Cefoxitin 30µg Disk 24 hours

HardyCHROM™ MRSA 24 hours	**Cefoxitin 30µg 24 hours**			**Percent Agreement**
	MRSA	**Non-MRSA***	**Total**	
MRSA	126	1**	127	Positive Percent Agreement – 93.3% (95% CI 89.6 – 98.4%)
Non-MRSA*	9	307	316	Negative Percent Agreement – 99.7% (95% CI 98.9 – 100%)
Total	135	308	443	

*****Organisms producing other types of non-*mec*A resistance were not evaluated on this medium (e.g. MOD-SA and BORSA).
** 1/1 Blood Agar Plate negative specimen was confirmed as MRSA positive by cefoxitin disk diffusion.

RECOVERY RATE

HardyCHROM™ MRSA was evaluated to determine the recovery rate (limit of detection (LoD)) of methicillin-resistant *Staphylococcus aureus*. Two ATCC® strains of MRSA (one strain with heterogeneous resistance and one strain with homogenous resistance) were evaluated for recovery on HardyCHROM™ MRSA. Sheep Blood Agar (BAP) plates were used to determine the concentration of organisms present in each dilution. At 10^3 CFU/ml, there was no discernable difference in recovery. Variable recovery was seen at lower concentrations.

REPRODUCIBILITY TESTING

Testing of HardyCHROM™ MRSA was evaluated at all three testing sites for three days in triplicate by three different operators using three different lots of media. The challenge strains were clinical isolates obtained from a private culture collection and included ten MRSA and ten MSSA strains. All isolates were coagulase positive and were characterized by PBP2' and cefoxitin testing. All of the isolates showed expected results.

Fresh suspensions of the well-characterized clinical strains were prepared in Tryptic Soy Broth at concentrations of approximately 10^5 to 10^6 for the MRSA strains and 10^6 to 10^7 for MSSA strains. 10 µl of these suspensions were used to inoculate HardyCHROM™ plates. A reproducibility rate of 100% for both inter-lot, operator, and testing intervals was achieved with the HardyCHROM™ MRSA media. At

each clinical study site, the sensitivity was 100% for the ten MRSA strains and 100% specificity for the ten MSSA strains.

CHALLENGE TESTING

Internal testing was performed using fifteen well characterized MRSA strains including isolates from USA100, USA200, USA300-0114 USA400, USA500, USA600, USA700, USA800, USA1000, and USA1100[7-12]. DNA restriction patterns were determined by pulsed-field gel electrophoresis.[12] Five additional MRSA strains representative of the prevalent HA-MRSA and CA-MRSA, and five MSSA strains (including Hypervirulent MSSA (NRS72)) were also tested) [7-12]. The MRSA strains were tested using a suspension containing approximately 10^5 to 10^6 CFU/ml and the MSSA strains were tested at a concentration of approximately 10^6 to 10^7. Ten µl of these suspensions were inoculated onto HardyCHROM™ MRSA. The sensitivity was 100% for the MRSA strains and 100% specificity for the MSSA strains.

INTERFERENCE STUDY

Commonly used transport devices were evaluated for potential interference of growth or the chromogenic reaction on HardyCHROM™ MRSA medium. In the analytical study the use of transport swabs containing rayon tipped plastic shaft swabs was evaluated. In the clinical study, rayon tipped plastic shaft swabs and nylon flocked Amies Eswabs were used with no observable differences in recovery. Transport media evaluated included Stuarts gel without charcoal, Stuarts Liquid, Amies Liquid, Amies Gel, and Amies Charcoal. No interference was noted with the use of different types of transport media. Nasal sprays which contain phenylephrine hydrochloride at 1% demonstrated an inhibitory effect on organism growth that is unrelated to medium performance. The presence of human blood or mucin did not affect the recovery of MRSA organisms.

CROSS REACTIVITY

Internal testing of other *Staphylococcus* and non-*Staphylococcus* organisms was conducted to determine if there was any cross reactivity when tested on HardyCHROM™ MRSA medium. A total of 121 strains were tested and included the following genera: *Acinetobacter, Burkholderia, Candida, Corynebacterium, Enterococcus, Enterobacter, Escherichia, Haemophilus, Klebsiella, Leuconostoc, Micrococcus, Moraxella, Neisseria, Proteus, Pseudomonas, Streptococcus* and *Staphylococcus*. *Staphylococcus* strains included MRSA, MSSA, and 20 different species of coagulase negative *Staphylococci* (including methicillin resistant *Staphylococcus epidermidis*). In the cross reactivity study, only *Corynebacterium jeikeium* produced a purple film in the first quadrant at 24 hours. At 48 hours, very small dark purple colonies were present but were only present in the first quadrant. *Staphylococcus intermedius* produced gray-blue colonies. All other organisms were inhibited.

MIXED INFECTION STUDY

A study was conducted using known concentrations of *E. coli* ATCC® 25922, *S. aureus* ATCC® 25923, and MRSA strains ATCC® 43300 and ATCC® 33591. Suspensions of the non-MRSA organisms were prepared at 10^8 and 10^9 and mixed with suspensions of the MRSA strains at the detection limit concentration (10^3 CFU/mL). No breakthrough of non-MRSA strains occurred at 24 or 48 hours and the MRSA strains were recovered at their LoD.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus aureus ATCC® 43300***	A	24hr	35 to 37ºC	Aerobic**	Growth; pink to magenta colonies
Staphylococcus aureus ATCC® 29213***	B	24-48hr	35 to 37ºC	Aerobic**	Inhibited
Staphylococcus epidermidis ATCC® 12228	B	24-48hr	35 to 37ºC	Aerobic**	Inhibited
Escherichia coli ATCC® 25922	B	24-48hr	35 to 37ºC	Aerobic**	Inhibited

*Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics software program HUGO™, for a description of inoculation procedures.

**Do not incubate in CO_2.

***Recommended strains for User Quality Control.

USER QUALITY CONTROL

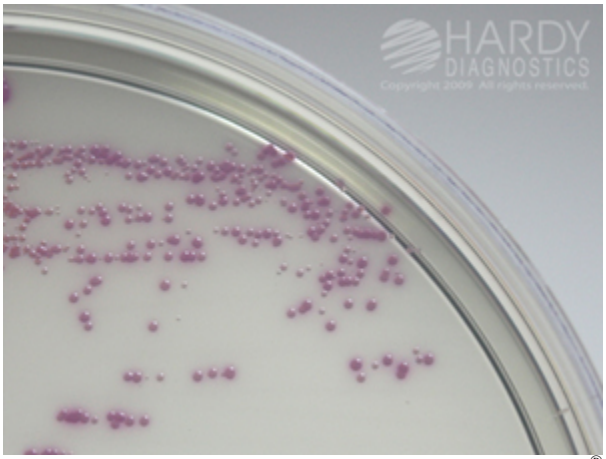
Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable) each week of use. Refer to the following keywords, in the Hardy Diagnostics software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

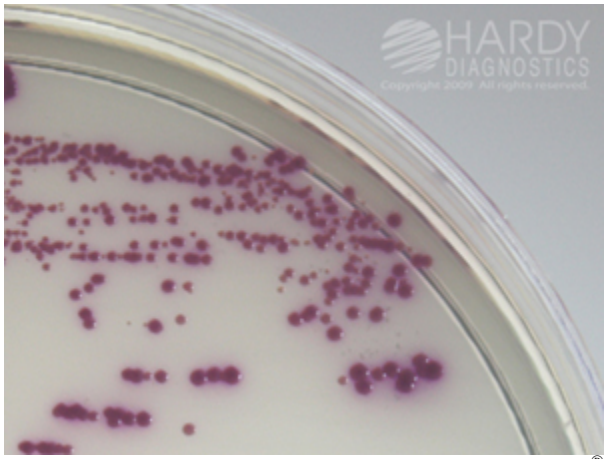
Standard microbiological supplies and equipment such as loops, other culture media, Coagulase Cryo™ (Cat. no. Z202), StaphTEX™ (Cat. no. ST50), swabs, applicator sticks, incinerators, and incubators, etc, as well as serological and biochemical reagents, are not provided.

PHYSICAL APPEARANCE

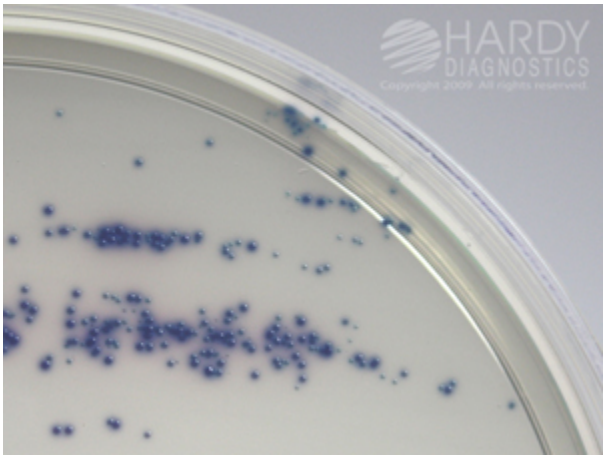
HardyCHROM™ MRSA should appear translucent and light amber in color.



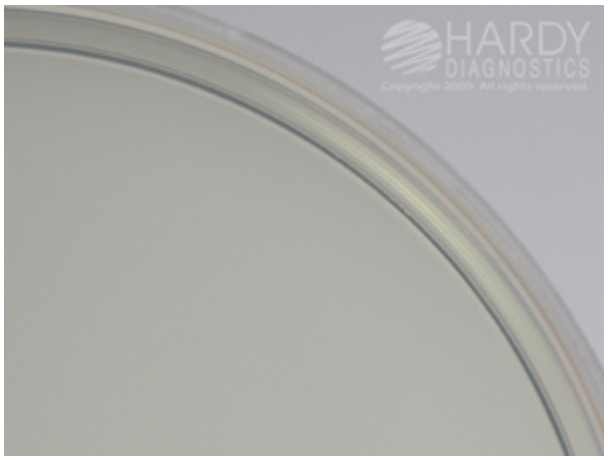
Methicillin-resistant *Staphylococcus aureus* (ATCC® 43300) growing on HardyCHROM™ MRSA (Cat. no. G307) showing pink colonies. Incubated aerobically for 24 hours at 35 to 37ºC.



Methicillin-resistant *Staphylococcus aureus* (ATCC® 43300) growing on HardyCHROM™ MRSA (Cat. no. G307) showing magenta colonies. Incubated aerobically for 48 hours at 35 to 37ºC.



Staphylococcus intermedius (clinical isolate) growing on HardyCHROM™ MRSA (Cat. no. G307) showing blue colonies. Incubated aerobically for 48 hours at 35 to 37ºC.



Uninoculated plate of HardyCHROM™ MRSA (Cat. no. G307).

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HARDY DIAGNOSTICS
1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · Florida

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