

Instructions for Use

HardyCHROM™ Staph aureus

Cat. no. G311	HardyCHROM™ Staph aureus, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J35	HardyCHROM™ MRSA/HardyCHROM™ Staph aureus, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

HardyCHROM™ Staph aureus is a chromogenic medium recommended for the isolation, differentiation, and enumeration of *Staphylococcus aureus* by colony color.

SUMMARY

Staphylococcus aureus is a gram-positive, coagulase-positive cocci that has been well documented as a human pathogen. *S. aureus* has also been implicated in nosocomial infections and food poisoning outbreaks. Many *S. aureus* strains produce enterotoxins that cause food poisoning when ingested. Food poisoning, bacteremia, pneumonia, toxic shock syndrome, and meningitis are some of the more serious infections that can be caused by *S. aureus*.

HardyCHROM™ Staph aureus allows for the rapid and reliable detection of *S. aureus* from both clinical and food specimens within 24 hours. Peptones in the medium supply the necessary nutrients. Selective agents inhibit the growth of gram-negative organisms, yeast, and some gram-positive cocci. Artificial substrates (chromogens) are broken down by specific microbial enzymes which release insoluble colored compounds. *S. aureus* uses only one of the chromogens and will produce deep pink to fuchsia colored colonies. Bacteria other than *S. aureus* may utilize the other chromogenic substrates and produce blue or turquoise colonies. If none of the substrates are utilized, natural or white colored colonies will be present. This medium can also be utilized in spread plate enumeration techniques.

FORMULA

Ingredients per liter of deionized water:*

Peptones	40.0gm
Sodium Chloride	25.0gm
Chromogenic Mixture	4.0gm
Selective Agents	1.0gm
Agar	15.0gm

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

Final pH 7.05 +/- 0.25 at 25°C

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°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.

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 handled observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

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

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Clinical Procedure

Specimen Collection: 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 onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.⁽²⁻⁵⁾

Method of Use: Allow the plates to warm 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 35-37 °C for 20 to 28 hours.

NOTE: When using HardyCHROM™ MRSA/ HardyCHROM™ Staph aureus biplate (cat. no. J35), inoculate HardyCHROM™ Staph aureus (side II) first, followed by HardyCHROM™ MRSA (side I) to prevent carryover of selective agents. Do not incubate biplate past 24 hours.

Industrial Procedure:

Specimen Collection: Consult listed references for information on specimen collection and processing of food, dairy, water samples and other materials of sanitary significance.⁽⁶⁻⁸⁾

The plates should be warmed to room temperature and the agar surface should be dry before inoculating.

Spread Plate Method:





1. Prepare serial dilutions in sterile diluent to obtain 30-300 CFU per plate.
2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.
3. Using a sterile spreader device (Cat. no. 174CS01), distribute the inoculum evenly over the agar surface.
4. Incubate plates aerobically for 20 to 28 hours at 35°C.

INTERPRETATION OF RESULTS

After incubation (20-28 hours), read plates against a white background.

Staphylococcus aureus will appear as smooth, deep pink to fuchsia colored colonies. Most other organisms, including *Staphylococcus epidermidis* will be partially to completely inhibited. Other organisms that may grow on HardyCHROM™ Staph aureus may appear as cream, blue, or colorless colonies. *Staphylococcus saprophyticus* will appear as turquoise colored colonies. Some gram-positive organisms other than *S. aureus* may appear as blue colonies.

Spread Plate Method: Following incubation, examine the plates for growth of *S. aureus*. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample; take into account the dilution factor. If duplicate plates were set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on this medium.⁽⁶⁻⁸⁾

Organism	Description	Photo	Color
<i>Staphylococcus aureus</i>	deep pink to fuchsia colonies		
<i>Staphylococcus saprophyticus</i>	turquoise colonies		

LIMITATIONS

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ Staph aureus.

Some non-*S. aureus* colonies may develop a light pink color after 48 hours. Do not incubate plates more than 24 to 28 hours. Some other staphylococcal strains may produce fuchsia colored colonies within 24 hours. Due to the nutritional variance among aberrant wild strains, rare strains (of *Staphylococcus aureus*) may show limited to no growth on HardyCHROM™. Coagulase testing or latex agglutination testing should be used to confirm results.

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, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing HardyCHROM™ Staph aureus at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Staphylococcus aureus</i> ATCC® 25923**	A	24hr	35°C	Aerobic	Growth; smooth, deep pink to fuchsia colonies
<i>Staphylococcus saprophyticus</i> ATCC® 15305**	A	24hr	35°C	Aerobic	Growth; turquoise colonies
<i>Staphylococcus epidermidis</i> ATCC® 12228	B	24hr	35°C	Aerobic	Partial to complete inhibition; light pink colonies may appear after extended incubation (48 hours)
<i>Enterococcus faecalis</i> ATCC® 29212	B	24hr	35°C	Aerobic	Partial to complete inhibition; blue

					colonies may appear
<i>Escherichia coli</i> ATCC® 25922**	B	24hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

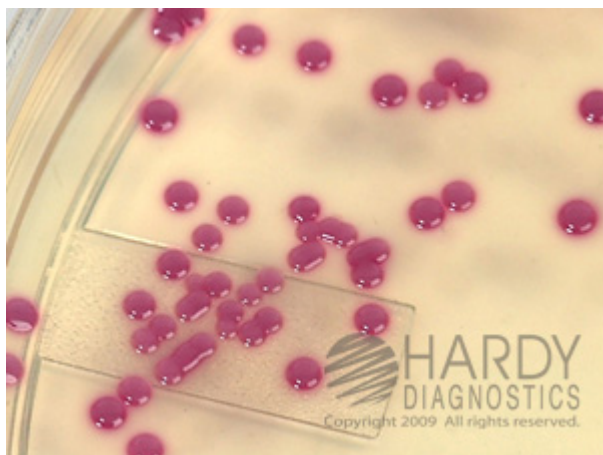
** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

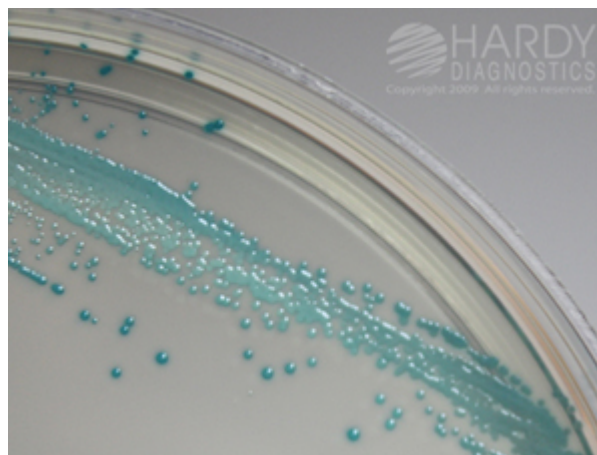
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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 certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

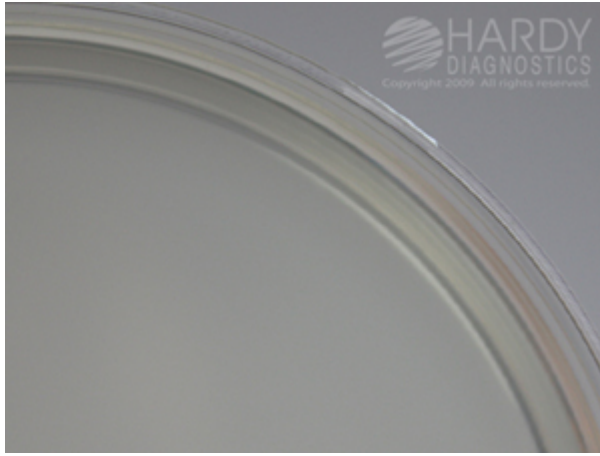
HardyCHROM™ Staph aureus should appear translucent, and light amber in color.



Staphylococcus aureus (ATCC® 25923) colonies growing on HardyCHROM™ Staph aureus (Cat. no. G311). Incubated aerobically for 24 hours at 35°C.



Staphylococcus saprophyticus (ATCC® 15305) colonies growing on HardyCHROM™ Staph aureus (Cat. no. G311). Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of HardyCHROM™ Staph aureus (Cat. no. G311).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
3. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*. J.B. Lippincott Company, Philadelphia, PA.
6. *Standard Methods for the Examination of Dairy Products*, 16th ed. 1992. APHA, Washington, D.C.
7. *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. 2001. APHA, Washington, D.C.
8. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*, 8th ed. AOAC, Arlington, VA.

ATCC is a registered trademark of the American Type Culture Collection.

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