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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

HARDYCHROM™ SALMONELLA SHIGELLA (SS) NOPRO AGAR

Cat. no. G327	HardyCHROM™ SS NoPro Agar, 15x100mm Plate, 21mL	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ SS NoPro (no-*Proteus*) Agar* is recommended for the selective isolation and differentiation of *Salmonella* and *Shigella* spp. from stool. HardyCHROM™ SS NoPro Agar is intended as a primary screening tool to distinguish *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria based on colony color, while inhibiting the growth and characteristic swarming of *Proteus* spp. The enhanced inhibition of *Proteus* reduces the expense involved in working up non-pathogens that could mimic enteric pathogens. Further species confirmation of suspect colonies via conventional or automated methods is recommended.

* Manufactured and sold in the US under license from Glycosynth Limited under U.S. Patent No.'s 7,323,488 B2, 7,384,763 B2, and 7,709,223 B2.

SUMMARY

Many formulations of culture media (such as HE, SS and XLD) have been developed to isolate and differentiate *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria. Most formulations incorporate common ingredients such as carbohydrates (especially lactose), pH indicators, and an indicator system for the detection of hydrogen sulfide. These media are made selective by the addition of bile salts and can also differentiate between *Salmonella*/*Shigella* and lactose-fermenting organisms. However, the problem has always been that colonies of non-lactose-fermenting organisms that are not pathogenic can appear similar in appearance to *Salmonella* and *Shigella* and must be subjected to further testing by using Triple Sugar Iron (TSI) Agar, Lysine Iron Agar (LIA), or Kligler Iron Agar (KIA). Screening of primary plates or secondary plating from enrichment broths often requires the inoculation of large numbers of secondary screening tubes and/or the use of costly automated identification systems in order to rule out false-positives.

The use of chromogenic substrates (chromogens) in media formulations has increased greatly in the last several years. Chromogens, when broken down by specific bacterial enzymes, will result in colored colonies. Previously, chromogenic formulations were available for *Salmonella* spp., but not for *Shigella* spp.

HardyCHROM™ SS NoPro Agar allows for the selective isolation and differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria. Based on colony color all species of *Salmonella* and *Shigella* can be readily distinguished from other non-lactose fermenting gram-negative bacteria. Differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic bacteria is accomplished by three mechanisms: chromogenic reactions, carbohydrate fermentation, and hydrogen sulfide production. HardyCHROM™ SS NoPro Agar provides better differentiation of colonies obtained from clinical samples and enrichment procedures, resulting in less secondary screening of isolates and less false-positive results. *Proteus* spp. will be completely to partially inhibited, thus preventing needless identification of false positives.

Bile salts and sodium deoxycholate allow for the selective nature of HardyCHROM™ SS NoPro Agar by inhibiting

gram-positive organisms. Additional selective agents are added to reduce the number of normal enteric bacteria and the formulation has been modified to prevent the growth and swarming of *Proteus* spp. This results in far fewer subcultures of lactose negative colonies for biochemical testing and faster detection of pathogens that may be masked by the growth of *Proteus* spp. Fermentable carbohydrates aid in the differentiation of enteric pathogens from delayed lactose fermenters. This reaction is visually enhanced by a pH indicator. The addition of ferric ammonium citrate and sodium thiosulfate enable the detection of H_2S, noted by the production of black centered colonies. Sodium thiosulfate serves as the sulfur source and ferric ammonium citrate is added as the indicator. Peptones in the medium supply the principle source of organic nitrogen in the form of amino acids and long-chained fatty acids. Patented chromogenic substrates are incorporated to enable the production of different colored compounds when degraded by specific bacterial enzymes.

FORMULA

Ingredients per liter of deionized water:*

Carbohydrates	20.0g
Peptones	8.0g
Sodium Thiosulfate	3.5g
Sodium Deoxycholate	1.5g
Ferric Citrate	1.0g
Chromogenic Mixture	0.5g
Selective Agents	0.15g
pH Indicator	0.01g
Agar	15.0g

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: 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: Consult listed references for information on specimen collection.[3-6,8] 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 the listed references for information regarding the processing of specimens.[3-6,8]

Method of Use: Allow the plates to warm to room temperature. The agar surface should be dry prior to inoculating to ensure well-isolated colonies.

Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. A less selective medium such as MacConkey Agar (Cat. no. G35) should also be inoculated. This increases the chance of recovery when the population of gram-negative organisms is low. It also provides indication of other organisms present in the specimen.

Incubate plates in an inverted position, protected from the light, aerobically at 35-37ºC. for 18-24 hours.

Examine plates for colonies showing typical morphology and color.

The use of enrichment procedures such as Selenite Cystine Broth (Cat. no. K69), GN Broth (Cat. no. K01 or K39), or Tetrathionate Broth (Cat. no. K65) is recommended when testing food or stool samples from food handlers.[8]

INTERPRETATION OF RESULTS

Most *Salmonella* serotypes will produce H_2S and the colonies will have a large black center with clear perimeter. Non H_2S producing *Salmonella* spp., including *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi and Choleraesuis, produce teal blue colored colonies.

Shigella spp. produce teal blue colored colonies; *S. flexneri*, *S. boydii*, and *S. dysenteriae* colonies generally have entire edges, while *S. sonnei* colonies may have entire or undulated edges (plasmid mediated). Note: *S. dysenteriae* may produce small, colorless colonies.

Note: Further species confirmation of suspect colonies via conventional or automated method is recommended. Teal color development may not be apparent at 18 hours of incubation in rare instances. Plates should be incubated a full 24 hours before being discarded as negative.

Other members of the *Enterobacteriaceae* will be partially to completely inhibited. However, if present, will produce:

- pink colonies, with or without purple centers (such as *Escherichia* spp., *Klebsiella* spp., *Citrobacter* spp., *Yersinia* spp., and *Enterobacter* spp.)

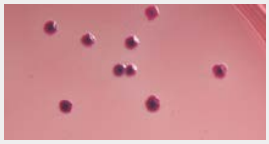
- dark blue colonies (*Citrobacter koseri*)

- dark blue, purple or violet colonies with clear or pink edges (such as *Serratia* spp., *Edwardsiella* spp. and *Morganella morganii*)

- pale pink or tan with small light to dark brown centers (*Proteus* spp.). Strains should be mostly inhibited.*

- small, blue colonies (such as *Hafnia alvei* and inactive *E. coli* (Alkalescens-Dispar) strains).

- brown or light pink colonies with blue/gray centers (*Providencia* spp.)

Organism	Description	Photo	Color
H_2S producing *Salmonella* spp.	Colonies with large black centers with clear perimeter		
Shigella spp. and non-H_2S producing *Salmonella* spp.	Teal blue colored colonies		
Escherichia spp., *Klebsiella* spp., *Citrobacter* spp., *Yersinia* spp., *Enterobacter* spp.	Pink colonies, with or without purple centers		
Hafnia alvei and inactive *E. coli* (Alkalescens-Dispar)	Small, blue colonies		

LIMITATIONS

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

Do not interpret colors of colonies on plates that have been incubated for longer than 24 hours.

Hafnia alvei and inactive *E. coli* produce blue colonies similar to *Salmonella* and *Shigella* spp. *H. alvei* and inactive *E. coli* colonies are more blue (less green) and are smaller than *Salmonella* and *Shigella* spp. Further testing is recommended.

Approximately 1% of *Shigella sonnei* strains are sucrose positive and may exhibit pink colonies with purple centers instead of teal blue colonies.

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ SS NoPro Agar.

Colonies of *Proteus* spp. should be inhibited on HardyCHROM™ SS NoPro Agar. In the event rare patient-specific isolates grow, colonies of *Proteus* spp. should be small non-swarming, pale pink in color, and may or may not produce dark centers that may resemble *Salmonella* or *Shigella* spp.

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 such as MacConkey AgarCat. no. G35), Selenite Cystine Broth (Cat. no. K69), GN Broth (Cat. no. K01 or K39), or

Tetrathionate Broth (Cat. no. K65), incinerators, incubators, 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	
Salmonella enterica ATCC® 14028	A	24hr	35°C	Aerobic	Growth; colonies with large black centers with a clear perimeter
Shigella sonnei ATCC® 9290	A	24hr	35°C	Aerobic	Growth; teal blue colonies
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Partial to complete inhibition; pink colonies with purple centers
Pseudomonas aeruginosa ATCC® 27853	B	24hr	35°C	Aerobic	Inhibited
Proteus mirabilis ATCC® 12453	A	24hr	35°C	Aerobic	Inhibited

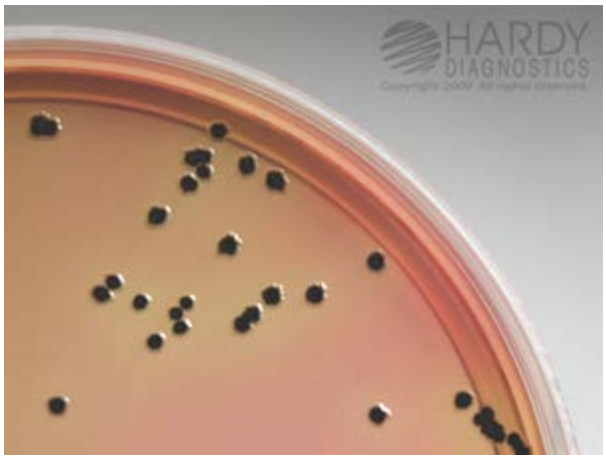
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

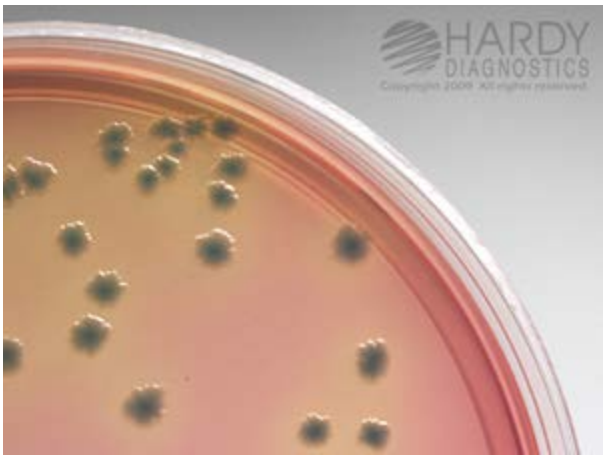
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

HardyCHROM™ SS NoPro Agar should appear clear, slightly opalescent, and dark pink in color



Salmonella enterica (ATCC® 14028) colonies growing on HardyCHROM™ SS NoPro Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35°C.



Shigella sonnei (ATCC® 9290) colonies growing on HardyCHROM™ SS NoPro Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35°C.

Escherichia coli (ATCC® 25922) colonies growing on HardyCHROM™ SS NoPro Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35°C.

Hafnia alvei (ATCC® 29926) colonies growing on HardyCHROM™ SS NoPro Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35°C.

Proteus mirabilis (ATCC® 43071) colonies inhibited on HardyCHROM™ SS NoPro Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35°C.

Uninoculated plate of HardyCHROM™ SS NoPro Agar. (Cat. no. G327).

REFERENCES

1. Gruenewald, R., et al. 1991. *J. Clin. Microbiol.*; 29:2354-2356.

2. Farmer, J.J., et al. 1985. *J. Clin. Microbiol.*; 21:46-76.

3. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*. American Society for Microbiology, Washington, D.C.

4. Murray, P.R. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

5. Forbes, B.A., et al. *Bailey and Scott's Diagnostic Microbiology*. C.V. Mosby Company, St. Louis, MO.

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

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

8. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*. APHA, Washington, D.C.

7. Centers for Medicare & Medicaid Services (CMS). Individualized Quality Control Plan (IQCP).

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

HDQA 2207F [D]