IFU

Instructions for Use

HARDYCHROM™ CAMPY AGAR

Cat. no. G339	HardyCHROM™ Campy Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ Campy Agar is recommended as a screening medium for the selective isolation and chromogenic differentiation of *Campylobacter* ssp. from direct stool cultures or from food or poultry samples. Some ingredients are very light sensitive. Store and incubate media in the dark.

SUMMARY

Campylobacter species have been recognized as a major cause of diarrheal disease in children and adults. Originally, these organisms were only associated with a variety of veterinary diseases. However, *Campylobacter* spp. have been characterized as among the top bacterial agents of human food borne gastroenteritis.(6) The organism may also be transmitted by contaminated food or water. Poultry is a primary reservoir of *Campylobacter* and studies show that their prevalence may be greater than 80% in commercial chicken carcasses.(2) Approximately 95% of human illnesses area associated with *Campylobacter jejuni*, followed by *Campylobacter coli* at 4%.(6) Other species are involved in only about 1% of infections.

HardyCHROM™ Campy Agar is composed of a highly nutritious basal medium containing vitamins, essential nutrients, salts, and growth factors to help support the growth of *Campylobacter* spp. The medium has been validated to selectively cultivate and chromogenically identify *C. jejuni*, *C. lari*, *C. coli*, and *C. fetus*. Selective agents act to suppress the growth of unwanted flora that may be present in the sample. Chromogens in the medium are utilized by the organism and cause *Campylobacter* colonies to appear red. Agar is the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Peptones	20.0g
Salts	5.0g
Growth Factors	1.0g
Yeast Extract	3.0g
Chromogenic Mixture	0.7g
Selective Agents	0.22g
Agar	13.0g

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store away from direct light 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.[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.

Fecal specimens are the preferred sample for isolating *Campylobacter* species from patients with gastrointestinal infections; however rectal swabs are acceptable for cultures. A transport medium, such as Cary-Blair or Campy-Thio medium, should be used if there is a delay of more than 2 hours to the lab, and for transport of rectal swabs. Specimens received in transport medium should be processed immediately or stored at 4ºC. until processed.[1,2]

Method of Use:

Direct Inoculation:

1. Swab: Inoculate a HardyCHROM™ Campy Agar plate using the four quadrant streak technique for maximum isolation.

2. Diarrheal Stool: Inoculate a HardyCHROM™ Campy Agar plate with three drops of stool and streak for isolation. At the same time, make a direct smear and look for small curved gram-negative bacilli and fecal leukocytes.

3. Solid Stool: Prepare a 1:10 suspension of stool by placing pea-sized amount into 5ml of physiological saline (0.85%). Vortex the sample. Inoculate a HardyCHROM™ Campy Agar plate with three drops of this suspension, and streak for isolation.

Indirect Inoculation:

1. Swab: Place the swab into an appropriate transport medium and refrigerate overnight.

2. Diarrheal Stool: Place five drops of the specimen approximately one centimeter below the surface of an appropriate broth medium (Cat. no. K128). Refrigerate overnight.

3. Solid Stool: Prepare a 1:10 suspension of stool by placing pea-sized amount into 5ml of physiological saline (0.85%). Vortex the sample. Place five drops of the specimen approximately one centimeter below the surface of an appropriate broth medium (Cat. no. K128). Refrigerate overnight.

4. Subculturing appropriate broth medium (Cat. no. K128): Place a pasteur pipet one inch below the surface of the broth medium and withdraw a large aliquot towards the surface. Place three drops onto a HardyCHROM™ Campy Agar plate and streak for isolation.

Sample Collection: Consult references for information concerning sample collection, pre-enrichment, and inoculation procedures.[1-5]

FDA-BAM Method:[4]

Pre-enrichment:

1. Place 11gm of food sample in 100ml of Bolton Broth. Alternatively, 25gm of food sample can be placed into 225ml of Bolton Broth (Cat. no. U83).

2. Mix the sample in the broth thoroughly to combine.

3. Incubate for 4 hours at 35ºC. Continue incubation for an additional 14 to 44 hours at 42 +/-1ºC.

USDA-FSIS Method:[5]

Pre-enrichment:

1. Place 30ml of Buffered Peptone Water (BPW) poultry rinsate into 30ml of Hunt Broth.

2. Mix to thoroughly combine.

3. Incubate for 48 +/- 2 hours at 42 +/-1°C.

USDA Direct Plating:

1. Prepare poultry samples by adding 325 +/- 32.5gm of raw poultry to 1625 +/- 32.5ml of BPW. Mix well in a stomacher or by hand massaging.

2. Inoculate plates with 1ml of the BPW sample using a sterile pipet (Cat. no. 1700).

Method of Use: Allow the medium to warm to room temperature prior to inoculation.

1. Streak the sample onto HardyCHROM™ Campy to achieve isolated colonies.

2. Incubate plates at 42 +/-1°C in a microaerophilic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 for 48 hours. If an appropriate microaerophilic incubator is not available, use a jar or pouch system such as the CampyGen gas generating system (Cat. no. CN025A) with a 2.5L jar (Cat. no. 16000). Alternatively, a smaller number of plates (from one to four 15x100mm plates) can be incubated using the CampyGen Compact (Cat. no. CN020C), pouches (Cat.no. AG020C), and sealing bars (Cat. no. AN005C). Do not disturb the microaerophilic environment during incubation, as

this can affect recovery of the organism.

3. Observe well isolated colonies for typical colony morphology and a red colony color.

INTERPRETATION OF RESULTS

Campylobacter colonies will appear red in color. To confirm colony identification, perform further confirmatory testing. Consult references for appropriate testing methods and interpretation of results.[1,3-5]

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.

Disturbing the microaerophilic atmosphere during log phase growth may affect recovery of the organism and cause a false negative result. Read plates after the full incubation period for best results.

Reactions observed on HardyCHROM™ Campy are not sufficient to speciate the organism. Further testing is required.

If HardyCHROM™ Campy was inoculated with a mixed sample, subculture suspected colonies to a non-selective medium to ensure culture purity prior to performing biochemical or confirmatory testing.

HardyCHROM™ Campy has been validated to selectively cultivate and chromogenically identify *C. jejuni*, *C. lari*, *C. coli*, and *C. fetus*. It is recommended red colonies grown on HardyCHROM™ Campy be further identified using a validated confirmatory method.

If further biochemical or confirmatory testing involves a colorimetric reaction, it is recommended users validate the test method by using a colony directly from HardyCHROM™ Campy Agar, and subculture the organism to a non-selective medium to ensure chromogens from the medium do not interfere with the results of the test.

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, CampyGen gas generating system (Cat. no. CN025A), 2.5L jar (Cat. no. 16000), CampyGen Compact (Cat. no. CN020C), pouches (Cat. no. AG020C), sealing bars (Cat. no. AN005C), 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	
Campylobacter jejuni ATCC® 33291	A	48hr	42°C	Microaerophilic***	Growth; red colony color
Escherichia coli ATCC® 25922	B	48hr	35°C	Aerobic	Inhibited
Proteus mirabilis ATCC® 12453	B	48hr	35°C	Aerobic	Inhibited

Candida albicans ATCC® 10231	B	48hr	35°C	Aerobic	Inhibited

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

** An incubation temperature of 42°C is recommended for samples containing mixed cultures to inhibit non-*Campylobacter* spp. For Quality Control testing pure isolates, an incubation temperature of 35°C may be used.

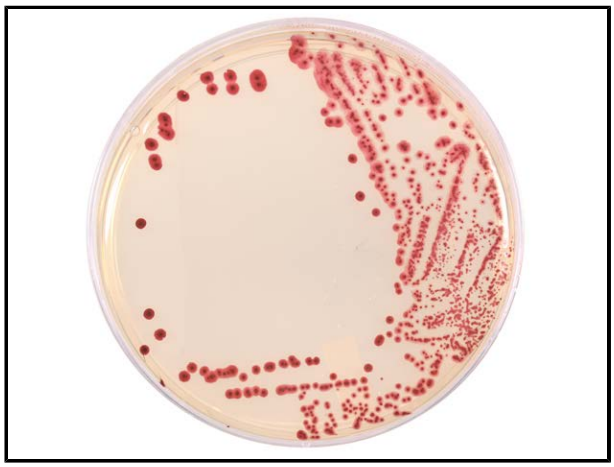
*** Atmosphere of incubation is enriched with 5% O_2, 10% CO_2, and 85% N_2.

USER QUALITY CONTROL

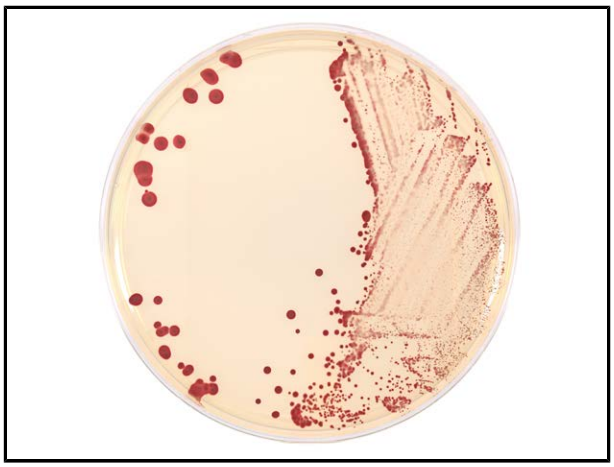
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory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

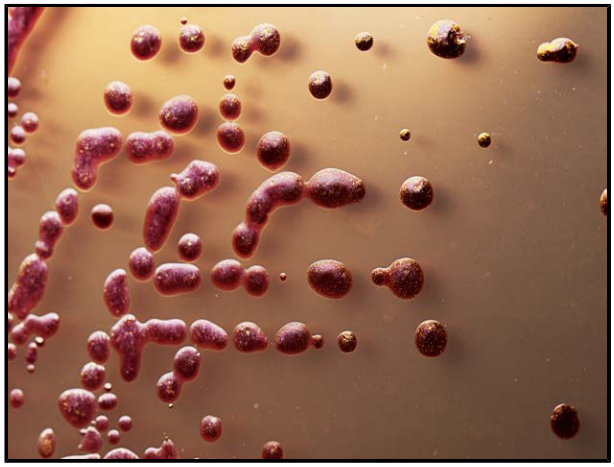
HardyCHROM™ Campy Agar should appear transparent and light amber in color; may have a slight precipitate.



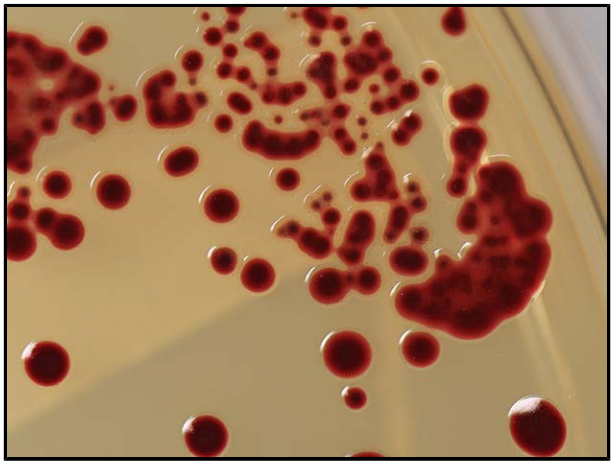
Campylobacter jejuni (ATCC® 33291) colonies growing on HardyCHROM™ Campy (Cat. no. G339). Incubated microaerophilically for 48 hours at 35ºC.



Campylobacter coli (ATCC® 33559) colonies growing on HardyCHROM™ Campy (Cat. no. G339). Incubated microaerophilically for 48 hours at 35ºC.



Campylobacter jejuni (ATCC® 33291) colonies growing on HardyCHROM™ Campy (Cat. no. G339). Incubated microaerophilically for 48 hours at 35ºC.



Campylobacter coli (ATCC® 33559) colonies growing on HardyCHROM™ Campy (Cat. no. G339). Incubated microaerophilically for 48 hours at 35ºC.

REFERENCES

1. Association of Official Analytical Chemists. *Official Methods of Analysis*, AOAC, Washington, D.C.

2. Oyarzabal, O.A., K.S. Macklin, J.M. Barbaree, and R.S. Miller. 2005. Evaluation of Agar Plates for Direct Enumeration of *Campylobacter spp.* from Poultry Carcass Rinses. *Appl. and Environ. Microbio.* 71(6):3351-3354.

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

4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* Arlington, VA http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

5. United States Department of Agriculture. Food Safety Inspection Service. *Microbiology Laboratory Guidebook.* MLG 41.04. USDA FSIS. Athens, GA.

6. Centers for Disease Control and Prevention. 2001. *Campylobacter* infections. https://www.cdc.gov/campylobacter/

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

HDQA 2207F [D]