anaeroGRO™
Pre-Reduced Anaerobic Culture Media

CAMPYLOBACTER SELECTIVE AGAR (CAMPY)

Cat. no. AG701	Campylobacter Selective Agar*, 15x100mm Plate, 18ml	1 plate/pouch

* All AnaeroGRO™ plated media is provided in standard 15x100mm monoplates or biplates. Each plate or set of plates is packaged in an oxygen-free gas flushed foil pouch containing a desiccant and an oxygen scavenger sachet.

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Campylobacter Selective Agar is recommended for the selective isolation of *Campylobacter jejuni* subsp. *jejuni* from stool specimens. Growth of normal fecal flora is inhibited on this medium.

SUMMARY

Campylobacter species are microaerophilic organisms that inhabit the gastrointestinal tract of various animals, including poultry, dogs, cats, sheep, and cattle. Microaerophilic organisms require an atmosphere consisting of 85% nitrogen, 5% oxygen, and 10% carbon dioxide for optimal growth. *C. jejuni* and *C. coli* are the most common *Campylobacter* species associated with gastrointestinal infection and are clinically indistinguishable. In fact, it is thought that approximately 5 to 10% of cases reported as *C. jejuni* in the U.S. are probably due to *C. coli*.

Campylobacter lari ATCC® has also been recognized as a cause of gastroenteritis, but less frequently than *C. jejuni*. *C. jejuni* continues to be the most common enteric pathogen isolated from patients with diarrhea. Symptoms of *C. jejuni* or *C. coli* infection usually include fever, abdominal cramping, and diarrhea that lasts for several days to more than one week. Symptomatic infections, such as gastroenteritis, are usually self-limiting and do not require antibiotic therapy, although relapses may occur in 5 to 10% of untreated patients. Deaths attributed to *C. jejuni* infection are uncommon.(1,6,10,11,13)

Campylobacteriosis is usually sporadic and tends to occur in the summer and early fall. Outbreaks are associated with ingestion of contaminated milk and water. Ingestion of improperly handled or under cooked food, primarily poultry products, raw milk, or contaminated water are common sources for human infections. It takes relatively few *Campylobacter* cells to cause illness or symptoms of gastroenteritis in humans. It is thought that the infective dose of *C. jejuni* ranges from 500 - 10,000 cells, depending upon the strain, damage to cells from environmental stresses, and susceptibility of the host.(1,7) Infants and young children are the most susceptible. Travelers to developing countries are also at risk for Campylobacteriosis.(1,2,6,10,11)

Early research by Dekeyser et al. on acute gastroenteritis using a filtration technique and a blood-containing selective medium isolated *Campylobacter jejuni* as the etiological agent.(4) Skirrow and others reported similar outcomes using blood-based selective media containing antimicrobics to suppress the growth of normal fecal

flora.[13] Later, Blaser et al. showed success in isolating *C. jejuni* using a Brucella Agar base supplemented with blood and selective agents.[3]

Peptones, yeast extract and other digests contained in these early formulations support the growth of *Campylobacter* spp. and supply a nutritious basal medium by providing nitrogenous compounds, carbon, sulfur, trace elements and complex B vitamins. The addition of blood, dextrose and other nutrients provides an energy source and further enhances the growth of cultures. The antimicrobial agents polymyxin B, trimethoprim, and vancomycin, along with incubation at 42ºC. in a microaerophilic environment, suppress the growth of normal fecal flora, thereby facilitating the isolation of *C. jejuni* from specimens.

AnaeroGRO™ Campylobacter Selective Agar is packaged in an oxygen-free, reduced state to prevent the formation of toxic oxidized by-products that may damage obligate anaerobes and inhibit the growth of more fastidious species. Culture media that is exposed to environmental oxygen leads to a build-up of reactive oxygen species (ROS) that initiate damaging free radical reactions, which inhibit the growth of anaerobic bacteria. Therefore, ingredients have been added to the AnaeroGRO™ media to neutralize the growth inhibiting toxic effects of peroxide and other reactive oxygen species (ROS) that may develop during the medium's brief exposure to oxygen after it is sterilized and before it is packaged in an oxygen-free environment.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	10.0gm
Peptic Digest of Animal Tissue	10.0gm
Soy Peptone	3.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Dextrose	1.0gm
L-Cysteine	0.5gm
Sodium Bisulfite	0.1gm
Vancomycin	10.0mg
Polymyxin B	2500.0IU
Trimethoprim	5.0mg
Laked Horse Blood	70.0ml
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating 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 quality control incubation times.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/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 document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1,2,5-11] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat, cold and oxygen exposure. If there is to be a delay in processing, it is recommended that the specimen be inoculated into an appropriate transport medium, such as Cary-Blair C&S Transport Vial (Cat. no. 28050510), and refrigerated until inoculation. Minimize specimen exposure to ambient oxygen levels in air.

Fecal specimens are the preferred sample for isolating *Campylobacter* species from patients with gastrointestinal infections; however rectal swabs are acceptable for culture. Cary-Blair (Cat. no. 4132BX) or Campy Thio Medium (Cat. no. K128) should be used as a transport medium 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 4ºCC. until processed.[6,11]

Method of Use: Open the AnaeroGRO™ pouch just prior to use and immediately apply rectal swabs or liquid fecal specimens directly to the agar surface in an area approximately 1 to 1.25 inches in diameter and streaked with a sterile inoculating loop to obtain isolated colonies. Incubate inoculated plates at 42 to 43ºC. in an anaerobic jar using a microaerophilic gas mixture (Cat. no. TN100) or CampyGen™ gas generating system (Cat. no. CN025A). For strain specific gas mixtures, consult appropriate reference materials for best practices. **Note:** *Campylobacter jejuni* is a microaerophile, not an obligate anaerobe.

Specimens cultured on selective media should also be cultured on non-selective media to obtain additional

information and to help ensure recovery of potential pathogens.

INTERPRETATION OF RESULTS

Examine plates at 24, 48, and 72 hours for growth. Colonies of *C. jejuni* subsp. *jejuni* are usually detected at 24 hours and produce two colony types: (1) small, raised, grayish-brown, smooth, and glistening with an entire translucent edge; or (2) flat, mucoid, translucent, grayish, with an irregular edge. A small percentage of strains may appear tan or faintly pink.[11] Colonies may spread over the entire surface of the agar, especially when isolated from fresh clinical specimens.

When examining plates at 24 hours, examine quickly and return plates to a reduced oxygen atmosphere promptly to avoid damaging cultures during log phase growth.

An oxidase test may be used to screen suspect colonies, since *C. jejuni* subsp. *jejuni* is oxidase positive. Indoxyl Acetate (Cat. no. Z111) is another rapid test that is useful for the identification of *Campylobacter*.

LIMITATIONS

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

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into a microaerophilic atmosphere (pouch or jar) to ensure optimal growth of microaerophilic bacteria.

C. jejuni is thermophilic and should be incubated at 42ºC. Otherwise, growth of colonies may be delayed.

Campylobacter species are not easily visualized with the safranin counterstain normally used in the Gram stain procedure; therefore, carbol fuchsin or 0.1% aqueous basic fuchsin (Cat. no. BF008) can be used as the counterstain, or extending the staining time of the safranin to at least 10 minutes can improve the intensity of the stain.[10,11]

Most *Campylobacter* species require a microaerobic atmosphere containing approximately 5% O_2, 10% CO_2, and 85% N_2 for optimal recovery. The concentration of oxygen generated in candle jars is not optimal for the isolation of *Campylobacter* spp. and should not be used.[11]

Certain *Campylobacter* species, such as *C. sputorum*, *C. concisus*, *C. mucosalis*, etc., may require hydrogen for primary isolation and growth.[11]

Due to the presence of dextrose in the medium, some weak oxidase reactions may occur. Testing should be performed on growth taken from a medium without dextrose if this phenomenon occurs.

Polymyxin B may be inhibitory to some strains of *C. jejuni* and *C. coli*.[11] Therefore, specimens cultured on selective media should also be cultured on non-selective media to obtain additional information and to help ensure recovery of potential pathogens.

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, transport media (Cat. no. 28050510 or 4132BX), incubators, incinerators, microaerophilic culture materials, such as gas generators (Cat. no. CN025A), compact systems (Cat. no. CN020C), pouches, (Cat. no. AG020C), sealing clips (Cat. no. AN005C), chambers, and jars (Cat. no. 16000), 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Campylobacter jejuni subsp. *jejuni* ATCC® 33291***	A	24-48hr	42°C	Microaerophilic**	Growth
Campylobacter fetus subsp. *fetus* ATCC® 33246	A	48hr	42°C	Microaerophilic**	Growth
Staphylococcus aureus ATCC® 25923	B	48hr	35°C	Aerobic	No growth; partial to complete inhibition
Enterococcus faecalis ATCC® 29212	B	48hr	35°C	Aerobic	No growth; partial to complete inhibition
Escherichia coli ATCC® 25922***	B	48hr	35°C	Aerobic	No growth; partial to complete inhibition
Proteus mirabilis ATCC® 12453	B	8hr	35°C	Aerobic	No growth; partial to complete inhibition

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

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

*** 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

AnaeroGRO™ Campylobacter Selective Agar should appear opaque to translucent, and medium red in color.

REFERENCES

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Microbiological Examination of Foods, APHA, Washington, D.C.

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

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