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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

CIN AGAR

Cat. no. G20	CIN Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J49	CIN Agar / MacConkey Agar with Sorbitol, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics CIN Agar is recommended for use in the selective and differential isolation of *Yersinia* and *Aeromonas* species from clinical specimens, environmental samples, and food sources.[6,7]

SUMMARY

Yersinia enterocolitica has been well documented as a causative agent of gastrointestinal infections that invades the intestinal mucosa and lymph nodes. It is a major cause of enteric illness in the northern United States, Canada, and Europe. It may occur either sporadically, or through food or water-borne outbreaks. However, infections have been found at extraintestinal sites as well. Other *Yersinia* spp. have also been implicated as human pathogens, but are found less frequently than *Y. enterocolitica*.

CIN (Cefsulodin, Irgasan, Novobiocin) Agar, originally developed in 1979 by Schiemann, is a highly selective medium designed to isolate *Yersinia enterocolitica*. The properties of this medium are based on selective chemical agents, antibiotics, dyes, and the basal medium. It is highly selective against the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Shigella sonnei* and *Streptococcus faecalis*.[6,7] The characteristic deep red center with a transparent margin, or "bull's-eye" appearance of *Yersinia* and *Aeromonas* colonies is important for identification, and is due to the presence of mannitol. *Y. enterocolitica* ferments the mannitol in the medium, producing an acid pH which gives the colonies their red color and the "bull's eye" appearance. Sodium deoxycholate, cefsulodin, irgasan, and novobiocin are added as selective agents. Altorfer found that by reducing the concentration of cefsulodin from 15.0 to 4.0mcg/ml, CIN Agar could also be used to selectively isolate *Aeromonas* spp., in addition to *Yersinia*.[8]

Studies have proved that CIN Agar is superior to SS (Salmonella-Shigella) Agar and MacConkey Agar in recovery rates of *Y. enterocolitica* from clinical specimens and food sources.[7]

FORMULA

Ingredients per liter of deionized water:*

Mannitol	20.0gm
Peptone	17.0gm
Proteose Peptone	3.0gm
Yeast Extract	2.0gm

Sodium Pyruvate	2.0gm
Sodium Chloride	1.0gm
Sodium Deoxycholate	0.50gm
Sodium Cholate	0.50gm
Neutral Red	30.0mg
Magnesium Sulfate	10.0mg
Irgasan®	4.0mg
Cefsulodin	4.0mg
Novobiocin	2.5mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.4 +/- 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: 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 appropriate references to determine correct collection procedures for the specimen to be tested (stool, food, etc.).[1-5]

Method of Use: Allow plates to warm to room temperature. Using standard microbiology procedures, inoculate the plates directly with stool, or with specimen from transport medium or a cold enrichment broth. Streak for isolation. Incubate at 35ºC. for 24 hours or 22-25ºC. for 48 hours. The lower temperature is recommended for primary isolation of *Yersinia* and will produce colonies with a more distinct "bull's-eye".

INTERPRETATION OF RESULTS

CIN Agar Colony Morphology: After 18-48 hours, colonies of *Y. enterocolitica* and *Aeromonas* spp. have a deep red center surrounded by a translucent outer zone.

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.

Citrobacter species, *Enterobacter agglomerans* , *Serratia liquefaciens* , *Y. frederiksenii* , *Y. intermedia* , and *Y. kristensenii* may grow on CIN Agar and resemble *Y. enterocolitica* ("bull's-eye" colony morphology), but are easily differentiated by biochemical tests.

Enterobacter cloacae and *Serratia marcescens* are not inhibited on CIN Agar. However, they usually appear as raised, mucoid colonies with diffuse, pink coloration.

Characteristic pigmentation is stronger and more complete at 25ºC. and 48 hours of incubation than at 35ºC. for 24 hours of incubation. It has been found that some *Yersinia* strains may be inhibited at 35ºC. The lower temperature is recommended for primary isolation.

Enterobacteriaceae other than *Yersinia* may grow on CIN Agar, especially *Citrobacter* species. *Serratia* and *Citrobacter* cannot always be reliably differentiated from *Yersinia* by colony morphology alone. Therefore, biochemical and serological tests are necessary for confirmation and complete identification of isolates.

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, other culture media, swabs, applicator sticks, incinerators, and 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	
Yersinia enterocolitica ATCC® 9610	A	24-48hr	25°C	Aerobic	Growth; red center, transparent border
Aeromonas hydrophila					Growth; red center,

ATCC® 7966	A	24-48hr	35°C	Aerobic	transparent border
Proteus mirabilis ATCC® 12453	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
Enterococcus faecalis ATCC® 29212	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
Pseudomonas aeruginosa ATCC® 27853	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

* 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

CIN Agar should appear clear, slightly opalescent, and reddish-purple in color.

Yersinia enterocolitica (ATCC® 9610) colonies growing on CIN Agar (Cat. no. G20). Incubated aerobically for 24 hours at 35ºC.

Aeromonas hydrophila (ATCC® 7965) colonies growing on CIN Agar (Cat. no. G20). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) growth inhibited on CIN Agar (Cat. no. G20). Incubated aerobically for 24 hours at 35ºC.

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. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

3. Tille, P., 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. Schiemann, D.A. 1979. Synthesis of a selective agar medium for *Yersinia enterocolitica.* J. Microbiol.; 25:1298.

7. Schiemann, D.A. 1982. Development of a two-step enrichment procedure for recovery of *Yersinia enterocolitica* from food. *Appl. Environ. Microbiol.*; 43:14.

8. Altorfer, Regine, et al. 1985. *Journal of Clinical Microbiology*; Vol. 22, No. 4, p. 478-480. American Society of Microbiology.

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Ordering Information

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

HDQA 2207F [D]