IFU

Instructions for Use

CRITERION™ YERSINIA SELECTIVE AGAR (CIN) BASE

Cat. no. C5390	CRITERION™ Yersinia Selective Agar (CIN) Base	119gm
Cat. no. C5391	CRITERION™ Yersinia Selective Agar (CIN) Base	500gm
Cat. no. C5392	CRITERION™ Yersinia Selective Agar (CIN) Base	2kg
Cat. no. C5393	CRITERION™ Yersinia Selective Agar (CIN) Base	10kg
Cat. no. C5394	CRITERION™ Yersinia Selective Agar (CIN) Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Yersinia Selective Agar (CIN) Base is recommended for use in the isolation and differentiation of *Yersinia* and *Aeromonas* species.[6,7]

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

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. *Y. enterocolitica* infection may occur either sporadically, 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 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 and sodium deoxycholate in the medium. *Y. enterocolitica* ferments the mannitol in the medium, producing an acid pH which gives the colonies their red color. The sodium deoxycholate is responsible for the "bull's-eye" phenomenon. It was demonstrated 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*

Gram weight per liter:	59.5gm/L

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
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

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light pinkish-beige.

Store the prepared culture media at 2-8ºC.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 59.5gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

5. Aseptically add 10ml of sterilized solution containing 4.0mg of cefsulodin and 2.5mg of novobiocin.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G20.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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	Incubation			Results

	Method*	Time	Temperature	Atmosphere	
Yersinia enterocolitica ATCC® 9610	A	24-48hr	25°C	Aerobic	Growth; red center, transparent border
Aeromonas hydrophila ATCC® 7966	A	24-48hr	35°C	Aerobic	Growth; red center, 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

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Yersinia Selective Agar (CIN) Base powder should appear homogeneous, free-flowing, and light pinkish-beige in color. The prepared media should appear clear, slightly opalescent, and reddish-purple in color.

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

HDQA 2207B [D]