IFU

Instructions for Use

CRITERION™ INDOLE NITRATE MEDIUM

Cat. no. C5870	CRITERION™ Indole Nitrate Medium	50gm
Cat. no. C5871	CRITERION™ Indole Nitrate Medium	500gm
Cat. no. C5872	CRITERION™ Indole Nitrate Medium	2kg
Cat. no. C5873	CRITERION™ Indole Nitrate Medium	10kg
Cat. no. C5874	CRITERION™ Indole Nitrate Medium	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Indole Nitrate Medium is recommended for the detection of indole production and nitrate reduction by microorganisms.

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

CRITERION™ Indole Nitrate Medium is a combined test medium for indole and or nitrate determination for bacteria other than the Enterobacteriaceae. The low agar concentration creates varying degrees of anaerobiosis in the media. As a result, this semisolid media satisfies the oxygen requirements for aerobes, as well as facultative and obligate anaerobes.

The indole test is a qualitative procedure for determining the ability of bacteria to produce indole. Casein peptone provides the source of tryptophan in the media. By producing the enzyme tryptophanase, certain microbes can deaminate tryptophan to indole. Indole is detected when it reacts with p-Dimethylamino-benzaldehyde (Kovacs Reagent) under acidic conditions to produce a red color, indicative of a positive reaction.[4]

The nitrate reduction test is a qualitative procedure for determining the ability of bacteria to reduce nitrate. Organisms which possess the enzyme nitroreductase vary in their ability to reduce nitrate. In the reaction, potassium nitrate is reduced to nitrite, which may then be further reduced to nitrogen gas or ammonia. The end product of nitrate reduction is dependent upon the bacterial species.[5]

The reduction of nitrate to nitrite is determined by the development of a red color complex upon the addition of Reagent A (sulfanilic acid solution, Cat. no. Z71) and Reagent B (N,N-dimethyl-1-naphthylamine, Cat. no. Z72). The sulfanilic acid reacts with nitrite to form a diazonium salt which then couples with N,N-dimethyl-1-naphthylamine to produce a red dye complex. Absence of a red color reaction indicates that the organism has further reduced nitrite to ammonia or nitrogen gas, or that unreduced nitrate is present, thus indicating the organism does not possess the nitroreductase enzyme.

If an organism does not possess the enzyme, nitrate will remain present in the medium. Application of Reagent C (zinc dust, Cat. no. Z73) will convert nitrate to nitrite to form a red-dye complex. This test reaction is considered negative for

nitrate reduction. If, however, the organism has reduced nitrate beyond nitrite to nitrogen gas, application of zinc dust will not produce a color change. The test is then considered positive for nitrate reduction.

FORMULA

Gram weight per liter:*	25.0gm/L
Pancreatic Digest of Casein	20.0gm
Disodium Phosphate	2.0gm
Glucose	1.0gm
Potassium Nitrate	1.0gm
Agar	1.0gm

Final pH 7.2 +/- 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 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 25.0gm 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.

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. K147.

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.

CRITERION™ Indole Nitrate Medium is not recommended for the determination of indole production by coliforms and other enterics as these organisms reduce nitrate to nitrite, hindering the indole reaction.

Test isolates must be from pure culture and 18-24 hours old.

Interpretation of nitrate reduction color reactions should be made immediately, as color reactions with a positive test may fade rapidly. To avoid false-negative nitrite reduction reactions, negative nitrite reactions must be verified by the addition of Reagent C to the medium.

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 Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Clostridium sordelli ATCC® 9714	A	24-48hr	35°C	Aerobic	Growth; nitrate positive, indole negative
Propionibacterium acnes ATCC® 29399	A	24-48hr	35°C	Aerobic	Growth; nitrate negative, indole positive

* 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™ Indole Nitrate Medium powder should appear homogeneous, free-flowing, and beige in color. The prepared media should appear opalescent, and light amber 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.

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Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207B [D]