

Instructions for Use

INDOXYL ACETATE DISKS

Cat. no. Z111	Indoxyl Acetate Disks	25 disks/vial
-------------------------------	-----------------------	---------------

INTENDED USE

HardyDisk™ Indoxyl Acetate Disks are used to determine the ability of certain bacteria to hydrolyze indoxyl acetate. Indoxyl Acetate Disks can be helpful in the rapid identification and differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species.^(5,7)

SUMMARY

Identification of *Campylobacter* spp. and members of the related genera *Helicobacter* and *Wolinella* require a variety of physiological and biochemical tests, which are laborious and time-consuming. Identification can also be difficult, in that, these organisms characteristically do not ferment or oxidize carbohydrate substrates.^(6,7) Catalase, hippurate hydrolysis, and susceptibility to cephalothin and nalidixic acid are performed routinely in many labs for identification of *Campylobacter* spp. However, due to the emergence of atypical strains, such as, catalase (-) / hippurate (-) *C. jejuni*, their usefulness for differentiation of *Campylobacter* spp. has been limited to some degree.⁽⁶⁾ With some of these species being well-established human pathogens, for example, *C. jejuni* and *C. coli* are frequently associated with gastrointestinal infection, and the continued addition of new species to these genera, it is important to develop more rapid, sensitive, and specific tests to isolate and identify those species and subspecies that are generally accepted as important human pathogens.⁽⁷⁾ Hardy Diagnostics Indoxyl Acetate Disks are a rapid and reliable method, useful in the differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species.

Bacterial hydrolases release indoxyl from the compound, indoxyl acetate. In the presence of air (O₂), indoxyl changes to indigo white and then into indigo.^(5,7) Indoxyl acetate hydrolysis (IAH) is a reliable, 5-30 minute test, and is easy to read and interpret. The disk method requires less time and interpretation than the tube method.⁽⁷⁾

Hodge, Boczyk, and Wat evaluated IAH for differentiation of *Campylobacter* spp. in 1990. They concluded the test to be a useful aid in the differentiation of *Campylobacter* spp. and for the identification of strains with aberrant phenotypic properties.⁽⁶⁾ Indoxyl acetate hydrolysis (IAH) has proven to be helpful in distinguishing negative *H. pylori* strains, associated with type B gastritis and peptic ulcer disease in humans, from positive *H. mustelae* strains. It is also useful in distinguishing negative *C. lari* (formerly *C. laridis*) and *C. fetus* subsp. *fetus*, from positive *C. coli* and *C. jejuni*.⁽⁷⁾

FORMULA

Each HardyDisk™ Indoxyl Acetate Disk is prepared by impregnating a solution of indoxyl acetate and acetone onto a 3/8 inch diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20°C. away from direct light. Product should not be used if there are any signs of

deterioration, discoloration, 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: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Studies have shown that the type of media used in obtaining the organism to be tested does not affect the outcome of the indoxyl acetate hydrolysis test.^(5,8)

Method of Use:

1. Allow disks to equilibrate to room temperature prior to use.
2. Place the disk on a glass slide or in an empty petri dish and add 1 drop of sterile water to the disk. Do not saturate the disk.
3. Using a wooden applicator stick or wire loop, inoculate the disk with a heavy inoculum (several colonies) from a pure, 18-72 hour culture of the test organism.
4. Incubate disks aerobically at room temperature for up to 30 minutes, and observe for blue to blue-green color development.

INTERPRETATION OF RESULTS

Positive: Blue to blue-green color development within 20 minutes indicates a positive reaction, and that indoxyl acetate hydrolysis has occurred. The appearance of a pale blue color in 10-30 minutes, indicates a weak positive reaction.^(5,7)

Negative: No color development within 30 minutes. See limitations.

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.

To distinguish hippurate-negative *C. jejuni* from *C. coli* strains, genetic methods are still the only reliable means for separating these species.⁽⁷⁾

Some *C. cinaedi* strains have shown weak positive reactions.⁽⁶⁾

Some positive isolates have been found that require up to 45 minutes to turn positive, compared to 15 minutes for the tube test method.⁽⁷⁾

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	
<i>Campylobacter jejuni</i> ATCC® 33291	E	within 30 min.	15-30°C	Positive; blue to blue-green color
<i>Campylobacter fetus</i> subsp. <i>fetus</i> ATCC® 27374	E	up to 30 min.	15-30°C	Negative; no color change

* 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

HardyDisk™ Indoxyl Acetate Disks are 3/8 inch (in diameter) filter paper disks and should appear white to light pink in color.



Showing positive (left disk) and negative (right disk) reactions for HardyDisk™ Indoxyl Acetate Disks (Cat. no. Z111).

Disks were aseptically placed in a sterile petri dish and moistened with a drop of deionized water. Growth from 72 hour cultures of *Campylobacter jejuni* (ATCC® 33291) and *Campylobacter fetus* subsp. *fetus* (ATCC® 27374) were applied to the left and right disks, respectively. The disks were incubated aerobically for 30 minutes. The blue color reaction was indicative as positive for indoxyl acetate hydrolysis.

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. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.
6. Donna S. Hodge, Alexander Borczyk. 1990. Evaluation of the Indoxyl Acetate Hydrolysis Test for the Diff. of *Campylobacters*. *J. Clin. Microbiol.* 28:1482-1483.
7. Tatjana Popovic-Uroic, Charlotte M. Patton, Mabel A. Nicholson, and Julia A. Kiehlbauch. 1990. Eval. of the Indoxyl Acetate Hydrolysis Test for Rapid Diff. of *Campylobacter*, *Helicobacter*, and *Wolinella* Species. *J. Clin. Microiol.* 28:2335-2339.
8. Charles K. Mills and Robert L. Gherna. 1987. Hydrolysis of Indoxyl Acetate by *Campylobacter* species. *J. Clin. Microbiol.* 25:1560-1561.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10495[B]

Manufactured for:



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: HardyDiagnostics.com

Email: TechnicalServices@HardyDiagnostics.com

[Ordering Information](#)

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.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207F [D]