



INDOXYL ACETATE DISKS

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

PRECAUTIONS

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

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⁾

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

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

USER QUALITY CONTROL

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.

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Manufactured for:

