

# Instructions for Use

## HardyDisk™ OPTOCHIN DIFFERENTIATION DISKS

<a href="#">Cat. no. Z7011</a>	Optochin Differentiation Disks	1 x 50 disks/cartridge
<a href="#">Cat. no. Z7015</a>	Optochin Differentiation Disks	5 x 50 disks/cartridge

## INTENDED USE

Hardy Diagnostics Optochin Differentiation Disks are recommended for use in the presumptive identification of *Streptococcus pneumoniae* from other alpha-hemolytic streptococci.

## SUMMARY

*Streptococcus pneumoniae* is found commonly in the human respiratory tract flora, as are other streptococci. When implicated in disease states and isolated onto blood agar for identification, the hemolytic pattern this organism gives is not distinguishable from other alpha-hemolytic streptococci. Thus, differentiation and identification must be performed via biochemical and serological testing.

Sensitivity to optochin (ethylhydrocupreine hydrochloride) is a well established phenomenon for *Streptococcus pneumoniae*.<sup>(5)</sup> A positive presumptive identification of *S. pneumoniae* is made when a well defined zone of inhibition results around the impregnated disk. Other alpha-hemolytic streptococci do not display this clear zone of inhibition when in the presence of optochin.

## FORMULA

Each disk is impregnated with a solution of ethylhydrocupreine hydrochloride.

## STORAGE AND SHELF LIFE

Storage: Upon receipt, store at -20 to 8°C. away from direct light. The disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

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. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism in pure culture.

### Method:

1. Using an inoculating loop, select three to four well-isolated colonies of the alpha-hemolytic organism to be tested. An 18-24 hour culture (either BHI Broth, Cat. no. R20 or TSB, Cat. no. R30) of isolated organism can also be used for testing.

2. Streak the isolate onto one-half of a TSA-5% sheep blood agar plate (Cat. no. A10) so as to obtain confluent growth.<sup>(8)</sup>

**Important:** Use of media other than TSA-5% sheep blood agar is not recommended, as false identification may result.

3. Using sterile forceps, place an optochin disk onto the inoculated surface of the agar.

4. Press disk gently with the sterile forceps or loop so that the disk adheres firmly to the agar surface.

5. Incubate the plate at 35 +/- 2.0°C. for 18-24 hours in 5-10% CO<sub>2</sub> enriched environment.<sup>(8)</sup>

6. If zone of inhibition is present, measure the diameter with a millimeter ruler or caliper.

## INTERPRETATION OF RESULTS

A presumptive identification for *S. pneumoniae* can be made if the alpha-hemolytic colony produces a zone of inhibition of 14mm or greater around the disk. Organisms producing smaller zone sizes should be tested for bile solubility. Organisms with questionable zone sizes (6-14mm) around the disk should be presumptively identified as a pneumococci only if it is bile soluble.<sup>(2)</sup>

A non-pneumococcal alpha-hemolytic streptococci should grow right up to and under the optochin disk.

## LIMITATIONS

It has been shown that other strains of alpha-hemolytic streptococci may show a slight susceptibility to optochin.

Optochin susceptibility is a presumptive test only. It is recommended that further biochemical tests be performed for complete identification.

Many isolates will show indeterminate zones on media other than TSA-sheep blood agar. Isolates showing the indeterminate zones would require further testing to identify them as *S. pneumoniae*.<sup>(8)</sup>

*S. pneumoniae* isolates should be incubated in a CO<sub>2</sub> enriched environment, as some isolates will grow poorly or not at

all aerobically.<sup>(8)</sup>

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, incinerators, CO<sub>2</sub> incubators, swabs, and culture media, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

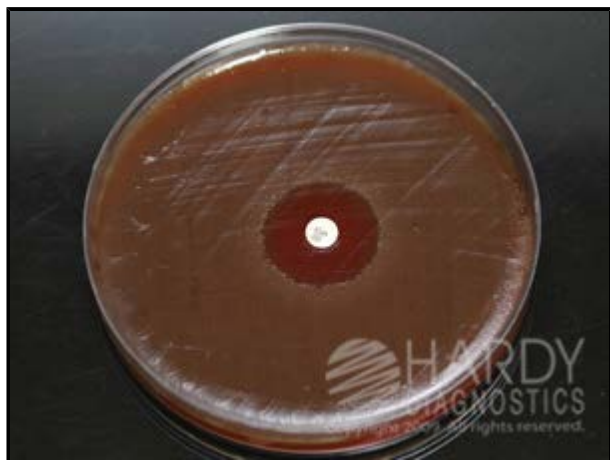
Test Organisms	Zone Diameter
<i>Streptococcus pneumoniae</i> ATCC <sup>®</sup> 6305	≥ 14mm
<i>Streptococcus pyogenes</i> ATCC <sup>®</sup> 19615	No zone

## 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™ Optochin Differentiation Disks are 6mm (in diameter) filter paper disks with the letters OP printed on both sides and should appear white in color.



### Optochin-sensitive (zone ≥ 14mm)

*Streptococcus pneumoniae* (ATCC<sup>®</sup> 6305) growing around a HardyDisk™ Optochin Differentiation Disk (Cat. no. Z7011) on Blood Agar (Cat. no. A10). Incubated in CO<sub>2</sub> for 24 hours at 35°C.



### Optochin-resistant (no zone)

*Streptococcus pyogenes* (ATCC<sup>®</sup> 19615) growing around a HardyDisk™ Optochin Differentiation Disk (Cat. no. Z7011) on Blood Agar (Cat. no. A10). Incubated in CO<sub>2</sub> for 24 hours at 35°C.

## REFERENCES

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4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
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7. Ragsdale, A.R. and Sanford, J.P. 1971. *Appl. Microbiol.*; 22:854-855.
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9. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services*. Subpart K - Quality System for Non-Waived Testing. 493;1200-1265.  
[www.cms.hhs.gov/clia](http://www.cms.hhs.gov/clia).

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

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