



HUGO™

HARDY USER GROUP OBSERVER ver 7.0

HardyDisk™ BACITRACIN DIFFERENTIATION DISKS

Cat. no. Z7021	Bacitracin Differentiation Disks	1 x 50 disks/cartridge
Cat. no. Z7025	Bacitracin Differentiation Disks	5 x 50 disks/cartridge

INTENDED USE

HardyDisk™ Bacitracin Differentiation Disks are used in the presumptive identification of group A, beta-hemolytic streptococci. The test differentiates group A streptococci from other Lancefield groups of hemolytic streptococci by inhibiting the growth of group A streptococci around a disk containing approximately 0.04units of bacitracin.

SUMMARY

Maxted reported a rapid diagnostic agent for the presumptive identification and differentiation of group A streptococci from other beta-hemolytic streptococci.⁽¹⁾ He tested over 3,0000 strains and found group A streptococci to be more sensitive to bacitracin than other beta-hemolytic strains. His findings showed that 99.0% of group A streptococci were inhibited and 95.3% of non-group A beta-hemolytic streptococci were not inhibited.⁽¹⁾

Levinson and Frank, and Streamer, et al., later confirmed Maxted's findings that group A streptococci proved more sensitive to bacitracin than other beta-hemolytic streptococci.^(2,3)

FORMULA

Each HardyDisk™ Bacitracin Differentiation Disk is prepared by impregnating approximately 0.04units of bacitracin onto a high quality 6mm diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at -20 to +8 degrees 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 applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

36 Months:	Z7021	HardyDisk™ Bacitracin Differentiation Disks
	Z7025	HardyDisk™ Bacitracin Differentiation Disks

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers of Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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.

Sterilize all biohazard waste before disposal.

Refer to the keyword "MSDS", in the Hardy Diagnostics software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Hemolytic isolates obtained from routine throat cultures:

1. Using a loop or swab, select 3-4 well isolated colonies of suspect beta-hemolytic organism from an 18-24 hour old culture and streak to the surface of a Blood Agar, 5% (Cat. no. A10) plate to obtain confluent growth.
2. Aseptically apply a HardyDisk™ Bacitracin Differentiation Disks in the center of the area containing the heavy inoculum.
3. Incubate inoculated media for 18-24 hours at 35 degrees C. in 5-10% CO₂. Observe for a zone of inhibition around the disk.

Direct inoculation (primary plating):

1. For early detection of *S. pyogenes* from throat cultures and other specimen types; it is acceptable to place a HardyDisk™ Bacitracin Differentiation Disk directly on the primary plating of the specimen. Either Blood Agar, 5% (Cat. no. A10), or Group A Beta Strep Agar (Cat. no. A72) plates may be used for this procedure.⁽¹²⁾
2. Aseptically apply a HardyDisk™ Bacitracin Differentiation Disk in the center of the area containing the primary or heavy inoculum.
3. Incubate inoculated media for 18-24 hours at 35 degrees C. in 5-10% CO₂. Observe for a zone of inhibition around the disk. If suspect beta-hemolytic, catalase negative colonies are found in an areas not directly adjacent to the disk, the colony must be subcultured to a Blood Agar plate and the Bacitracin Disk testing repeated. (See procedure listed above for testing beta-hemolytic isolates.)

INTERPRETATION OF RESULTS

Any zone of inhibition that extends beyond the edge of the disk is regarded as susceptible to bacitracin. It is, therefore, considered positive for a presumptive identification of group A streptococci.

LIMITATIONS OF THE PROCEDURE

Due to differences in zone sizes resulting from different concentrations of bacitracin, differential disks (0.04units), as opposed to sensitivity disks (10units), are used in the test.

When testing isolates, a light inoculum may result in false zones of inhibition, so it is important that an inoculum resulting in confluent growth be used.

If direct plating is done, the disk must be placed in the primary inoculum area. If hemolytic colonies are seen in an area not adjacent to the disk, the organism needs to be re-tested following procedures used for isolates.

When differentiating streptococci, some group A streptococci may not show a zone of inhibition if an excessive inoculum is used. It has been reported that 6% of group B and 7.5% of groups C and G streptococci may produce zones of inhibition (false-positive result).⁽¹¹⁾ Some streptococci may not grow in the absence of CO₂.

It is recommended that biochemical and/or serological tests such as latex agglutination (Cat. no. PL030HD), be performed on colonies from pure culture for complete identification.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, incinerator, incubators, and culture media, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Known positive (group A streptococci) and negative (non-group A streptococci) controls should be used to monitor the accuracy of the disks and inoculum.

Test Organisms

Streptococcus pyogenes
ATCC® 19615

Streptococcus agalactiae
ATCC® 13813

Results

Zone of inhibition

No zone of inhibition

User Quality Control

Check for signs of contamination and deterioration. It is recommended that each new lot or shipment of disks be tested with known positive and negative controls.^(7,8) Refer to the following keywords, in the Hardy Diagnostics software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also, see listed references for more information.⁽¹⁻⁴⁾

Physical Appearance

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



Bacitracin-sensitive (zone of inhibition).

Streptococcus pyogenes (ATCC® 19615) inhibition zone around a HardyDisk™ Bacitracin Differentiation Disk (Cat. no. Z7021). Incubated in CO₂ on Blood Agar (Cat. no. A10) for 24 hours at 35 deg. C.



Bacitracin-resistant (no zone of inhibition).

Streptococcus agalactiae (ATCC® 13813) growing around a HardyDisk™ Bacitracin Differentiation Disk (Cat. no. Z7021). Incubated in CO₂ on Blood Agar (Cat. no. A10) for 24 hours at 35 deg. C.

REFERENCES

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

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[Ordering Information](#)

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