BILE SPOT REAGENT

Cat. no. Z61	Bile Spot Reagent	15ml

INTENDED USE

Hardy Diagnostics Bile Spot Reagent is recommended for use in presumptively differentiating bile-soluble *Streptococcus pneumoniae* from bile-insoluble alpha-hemolytic *Streptococcus* spp. The bile solubility test may be performed using a cell suspension or by applying the Bile Spot Reagent directly to the colony.

SUMMARY

The bile solubility test is a qualitative procedure for determining the ability of bacterial cells to lyse in the presence of bile salts under specific conditions of time and temperature. The test is primarily used to differentiate bile-soluble *Streptococcus pneumoniae* from bile-insoluble alpha-hemolytic streptococci. The working mechanism of the test is not clearly understood, however, one theory is that the bile salts facilitate lysis of pneumococcal cells by activating an intracellular autolytic enzyme.

REAGENT FORMULA

Ingredients per liter of deionized water:*

Sodium Deoxycholate	100.0gm

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30ºC. Products should not be used if there are any signs of deterioration or if the expiration date has passed. Storage of the reagent at cool temperatures can cause it to thicken. Warm the reagent bottle in a 37ºC incubator to liquify the reagent before use.

The expiration dating 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 quality control incubation times.

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 *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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

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 organisms.

Test Tube Method:

1. Dispense 1ml of sterile 0.85% saline into a small test tube.

2. Prepare a heavy suspension of the organism in the saline. Shake or vortex to form a uniform suspension.

3. Divide the suspension into two tubes (0.5ml each), one labeled "TEST," the other labeled "CONTROL."

4. Dispense 10 drops of Bile Spot Reagent into the tube marked "TEST". Add 0.5ml of saline to the tube marked "CONTROL". Gently mix each tube.

5. Incubate the tubes for 3 hours at 35ºC, checking hourly.

Direct Plate Method:

1. Place a drop of Bile Spot Reagent near a suspected 18-24 hour old colony, gently roll the drop over several representative colonies. Take care not to dislodge the colonies.

2. Keep the plate right side up and incubate at 35ºC for 30 minutes.

INTERPRETATION OF RESULTS

Test Tube Method: Examine the "TEST" tube for clearing or loss of turbidity as compared with the "CONTROL" tube. Bile solubility is demonstrated as a clearing or loss of turbidity, relative to the "CONTROL" tube within three hours.

Direct Plate Method: Bile solubility is demonstrated as a disintegration of the colony and/or the appearance of a hemolytic zone in the medium at the sight where the colony was located within thirty minutes. Insolubility is demonstrated when there is no change in the integrity of the colony within 30 minutes.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Bile solubility is used only to differentiate *Streptococcus pneumoniae* from other alpha-hemolytic streptococci.

Additional biochemical testing using pure culture is recommended for complete identification. 86% of pneumococcal strains will lyse completely.

There are variations in the bile solubility of *S. pneumoniae* possible due to the loss of virulence factor.

Normal autolysis of *S. pneumoniae* may be inhibited by a high concentration of bile salts. Evaporation may cause the reagent to become more concentrated, thus affecting the test.

The bile solubility test is not always reliable since old cultures lose the active enzyme. Therefore, colonies resembling *S. pneumoniae* which are not bile-soluble should be further identified using another method.

When performing the bile solubility tube test using saline or unbuffered broth, it is essential to adjust the pH to neutral before adding the reagent in order to avoid false-negative reactions.

When testing using the plate method, care must be taken not to dislodge the colony being tested, thus leading to false-positive results. Place a drop of the Bile Spot Reagent near the colony and roll over several representative colonies by tilting the plate. If the direct plate is difficult to interpret the test should be repeated using the tube method.

Storage of the reagent at cool temperatures can cause it to thicken. Warm the reagent bottle in a 37ºC incubator to liquefy the reagent before use.

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, culture media, test tubes, incinerator, incubators, pasteur pipets, as well as serological and biochemical reagents, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

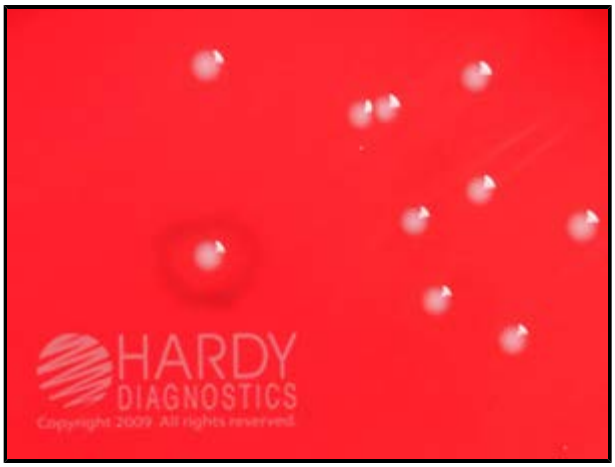
Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Streptococcus pneumoniae ATCC® 6305	A	24hr	35°C	Aerobic	Growth; colonies dissolve after adding reagent and incubating for 30min at 35°C
Enterococcus faecalis ATCC® 29212	A	24hr	35°C	Aerobic	Growth; colonies dissolve after adding reagent and incubating for 30min at 35°C

USER QUALITY CONTROL

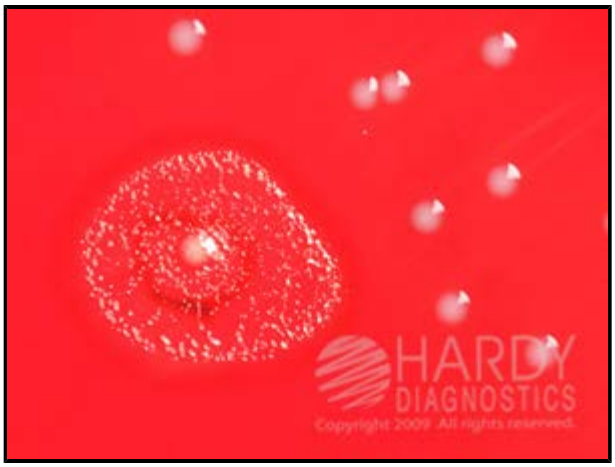
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Bile Spot Reagent should appear clear, and very light amber in color.



Enterococcus faecalis (ATCC® 29212) colony (incubated aerobically for 24 hours at 35 deg. C) before incubation with Bile Spot Reagent (Cat no. Z61). Incubated aerobically for 30 min. at 35ºC.



Enterococcus faecalis (ATCC® 29212) colony after incubation with Bile Spot Reagent (Cat no. Z61). Incubated aerobically for 30 min. at 35ºC.

Streptococcus pneumoniae (ATCC® 6305) colony before incubation with Bile Spot Reagent (Cat no. Z61). Incubated aerobically for 30 min. at 35ºC.

Streptococcus pneumoniae (ATCC® 6305) colony after incubation with Bile Spot Reagent (Cat no. Z61). Reagent was dropped within the black circle. Incubated aerobically for 30 min. at 35ºC.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. 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.

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.

6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria,,* Lipincott Williams & Wilkins, Philadelphia, PA.

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