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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

COAGULASE CRYO™

Cat. no. Z202	Coagulase Cryo™, 2ml Cryogenic Vial, 0.5ml	20 vials/pkg

INTENDED USE

Hardy Diagnostics Coagulase Cryo™ is used to perform the coagulase test from isolated colonies. The coagulase test is used for the identification of *Staphylococcus aureus*.

SUMMARY

The Coagulase Cryo™ contains frozen rabbit plasma which tests for the production of bacterial coagulase. Coagulase is a heat stable enzyme mainly found in *Staphylococcus aureus* and is used to differentiate *S. aureus* from other commonly isolated staphylococci. Two forms of coagulase exist: one is bound to the cell, and the other is excreted from the cell as an enzyme. Bound coagulase, also called "clumping factor," acts directly on the fibrinogen in plasma and causes the bacteria to clump. When the coagulase is released as an enzyme from the organism, also called "free coagulase," it converts prothrombin to a product that then acts on fibrinogen in the plasma to form a fibrin clot.[4]

The two main methods for determining the presence of coagulase, and thus *S. aureus*, are the tube coagulase test and slide coagulase test. These tests are used to identify pathogenic staphylococci. The *Staphylococcus* species that are capable of producing coagulase include *S. aureus* (potentially pathogenic in humans and animals) and the animal isolates *S. intermedius* and *S. hyicus*.[1]

The slide coagulase test has an approximate 96% agreement with the tube coagulase test.[4] However, the slide agglutination technique can occasionally generate false-positives results. This is due to the fact that some strains such as *S. lugdunensis* and *S. schleiferi* subsp. *schleiferi* produce clumping factor resulting in a positive slide test only. The more specific tube coagulase method can be used to differentiate these species from *S. aureus*.[4] In addition, spontaneous agglutination may occur on the slide agglutination test when rough cultures are used. When the slide test is employed, all negative slide reactions must be confirmed by the tube test.

FORMULA

Hardy Diagnostics Coagulase Cryo™ contains frozen rabbit plasma with EDTA.

STORAGE AND SHELF LIFE

Storage: **Upon receipt store -20 to -2ºC**, away from direct light. Product can be shipped without refrigeration if transit time is less than one week. Do not thaw and refreeze more than once. Media should not be used if there is any sign of deterioration, or if the expiration date has passed. Product is light and temperature sensitive. Protect from light and excessive heat.

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

Tube Coagulase Method:

1. Remove Coagulase Cryo™ from freezer and thaw to room temperature. Remove only the number of Coagulase Cryos™ that are required for testing that day, to protect the vials from excessive thawing and refreezing.

2. Using a sterile 1ml serological pipet (or transfer pipet), add approximately 0.05ml (1-2 drops) of an overnight broth culture (BHI or TSB) to the vial. Alternatively, using a pure, isolated culture that is less than 24 hours old, inoculate the Coagulase Cryo™ by emulsifying one loopful (2-4 colonies) of bacteria into the liquid.

3. Incubate the inoculated tube at 35-37ºC. for 1 to 4 hours. Negative tests at 4 hours should be held at room temperature for a total of 24 hours before reporting results.[2,3]

Note: Prolonged incubation at 35-37ºC. may cause some strains to produce fibrinolysin, which will break up the clot resulting in a false-negative reaction.[3] Incubation at room temperature after the initial four hours will prevent fibrinolysin from forming.

Slide Coagulase Test:[2]

1. Remove Coagulase Cryo™ from freezer and thaw to room temperature. Remove only the number of Coagulase Cryos™ that are required for testing that day, to protect the vials from excessive thawing and refreezing.

2. Place a drop of coagulase plasma on a clean, dry glass slide.

3. Place a drop of distilled water or saline near the drop of plasma as a control.

4. With a sterile loop or wooden stick, emulsify an amount of the pure isolated colony being tested into each drop, inoculating the water or saline first. Try to create a smooth suspension.

5. Observe for clumping in the coagulase plasma and a homogenous suspension in the control. Clumps that will not mix uniformly into coagulase plasma indicate a positive test whereas a uniform smooth suspension is indicative of a negative test. Clumping in both tests indicate that the organism autoagglutinates and is unsuitable for the slide coagulase test. When autoagglutination is observed, the tube coagulase test should be employed as an alternative to the

slide agglutination test. All negative slide tests should be confirmed using the tube test.

INTERPRETATION OF RESULTS

Tube Coagulase Test:

Results should be read at 4 hours. A positive test for coagulase production results in a clotting or gelling of the rabbit plasma. Any degree of clotting is a positive test.

Results can be reported across a range 0 to 4+, 0 meaning the plasma remained liquid (no coagulase activity) and 4+ meaning the plasma completely hardened (the consistency of an agar) due to strong coagulase activity.

All "0" results after 4 hours should be held at room temperature for a total of 24 hours incubation.[2,3]

Slide Coagulase Test:[2]

Clumps that will not mix uniformly into coagulase plasma represent a positive slide coagulase test and are indicative of *S. aureus*. A negative reaction is recorded when colonies mix smoothly into solution. Clumping in both the coagulase and control indicate that the organism autoagglutinates and is unsuitable for the slide coagulase test. When autoagglutination is observed, the tube coagulase test should be employed as an alternative to the slide agglutination test.

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.

The coagulase test can be used in the presumptive identification of *Staphylococcus aureus*. Coagulase-positive organisms should be tested for catalase activity and examined by gram stain to determine morphology and gram reaction. The rare isolates, *S. intermedius* and *S. hyicus* can also produce a positive coagulase test.

Tube Coagulase Test:

When checking the results of the tube coagulase test, tubes should be observed hourly during the first four hours of incubation. Some strains of *S. aureus* produce fibrinolysin which may lyse clots formed earlier. If the tubes are not read until 24 hours of incubation, reversion to a false-negative might result.

A flocculent or string like precipitate should not be considered a true clot, and should be reported as a negative result. Negative tubes must be held overnight because some *S. aureus* strains require longer than 4 hours to form a clot. Incubation beyond four hours must be performed at room temperature to prevent the production of fibrinolysin.[2,3]

Avoid shaking or agitating the Coagulase Cryo™ while reading the test.

Slide Coagulase Test:

The slide agglutination technique may lead to false-positives, since some strains such as *S. lugdunensis* and *S. schleiferi* subsp. *schleiferi* produce clumping factor resulting in a positive slide test and a negative tube coagulase test. In addition, spontaneous agglutination may occur when rough cultures are used. When the slide test is employed, all negative slide reactions must be confirmed by the tube test.[2]

A positive slide coagulase test result is valid only for strains of *Staphylococcus* spp. that have tested negative for autoagglutination. Autoagglutinating strains of staphylococci require an alternative method for testing for *S. aureus* such as the tube coagulase test.[4]

It is not recommended that colonies from high-salt containing agars, such as Mannitol Salt Agar, be used with the slide coagulase test.[4]

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, swabs, applicator sticks, other culture media, 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	Atmosphere	
Tube Coagulase Method:					
Staphylococcus aureus ATCC® 25923	E	4hr, 24hr	35°C 15-30°C	Aerobic	Clumping;coagulase-positive
Staphylococcus epidermidis ATCC® 12228	E	4hr, 24hr	35°C, 15-30°C	Aerobic	No clumping; coagulase-negative

* 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

Coagulase Cryo™ rabbit plasma should appear clear, and slightly amber in color.

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. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology* . J.B. Lippincott Company, Philadelphia, PA.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. *Commission on Laboratory Accreditation , Laboratory Accreditation Program Microbiology Checklist* . College of American Pathologists. Rev. 9/30/2004.

6. 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.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]