COAGUSTAPH™

Cat. no. Z003	CoaguStaph™, Coagulase Plasma with EDTA	6 x 3ml
Cat. no. Z020	CoaguStaph™, Coagulase Plasma with EDTA	6 x 20ml

INTENDED USE

Hardy Diagnostics CoaguStaph™ is used to perform the coagulase test. The coagulase test is used for the identification of *Staphylococcus aureus*.

SUMMARY

The CoaguStaph™ contains lyophilized rabbit plasma which tests for the production of 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] The slide agglutination technique, however, can occasionally generate false-positive 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 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

CoaguStaph™ contains lyophilized rabbit plasma with EDTA.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC., away from direct light. Once reconstituted, CoaguStaph™ can be stored at 2-8ºC. for up to 5 days or aliquot into 0.5ml amounts and stored at -20ºC. for up to 30 days. Reconstituted plasma should be frozen immediately after use. Do not thaw and refreeze hydrated CoaguStaph™. This product 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 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.

Reconstituted plasmas, if kept uncontaminated, retain their activity for five days when stored at 2-8ºC. and up to 30 days when aliquoted and stored at -20ºC., not exceeding the expiration date on the label.

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." The "Guidelines for Isolation Precautions" is available from the Centers for 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 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.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Tube Coagulase Method:

1. Reconstitute each vial of CoaguStaph™ using sterile deionized water as follows:

    Z003: 3ml of sterile water (approximately 6 tests)

    Z020: 20ml of sterile water (approximately 40 tests)

2. Aliquot 0.5ml of reconstituted CoaguStaph™ into sterile test tubes using a sterile pipette. Store CoaguStaph™ that will not be used immediately at a temperature below 20ºC. for 30 days.

3. Using a culture that is less than 24 hours old, inoculate the CoaguStaph™ by emulsifying one loopful (2-4 colonies) of bacteria from a non-inhibitory agar plate into the tube of plasma.

4. 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.

5. Read by gently tilting the tube while observing for clotting of plasma.

Slide Coagulase Test:[2]

1. Reconstitute each vial of CoaguStaph™ plasma using sterile deionized water as follows:

    Z003: 3ml of sterile water (approximately 6 tests)

    Z020: 20ml of sterile water (approximately 40 tests)

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 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 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.

INTERPRETATION OF RESULTS

Tube Coagulase Test:

Results should be read at 4 hours. A positive test for coagulase production results in a clotting 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

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 catalase test.

Additional biochemical tests are recommended for complete identification.

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 reconstituted CoaguStaph™ 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]

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological equipment such as inoculating loops, sterile tubes, sterile pipets, sterile deionized water, incinerators, incubators, slides, other culture media, 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 Certificates of Analysis (CofA). The following organisms 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	35°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 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

CoaguStaph™ should appear as a white-beige powder cake.

Showing positive (upper tube) and negative (lower tube) coagulase tests.
Two sterile test tubes each received a 0.5mL aliquot of reconstituted CoaguStaph™ (Cat. no. Z003). A loopful of *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 12228) from 18 hour cultures on TSA (Cat. no. G60) were emulsified in the upper and lower tubes, respectively. The tubes were incubated aerobically for four hours at 35 deg. C. Observable clumping was indicative of a positive coagulase test. Do not use cultures that are more than 24 hours old. Do not use cultures from an inhibitory medium. All negative tests should be held at room temperature for a total of 24 hours before recording results.

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. College of American Pathologists (CAP). Proposed CAP Checklist Requirements for IQCP. Northfield, IL.

6. Department of Health and Human Services. Centers for Medicare and Medicaid Services (CMS). Appendix C - Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services (Clinical Laboratory Improvement Amendments (CLIA)). Baltimore, MD.

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

HDQA 2207F [C]