

Bactident® Coagulase

For the detection of the enzyme coagulase developed by staphylococcus aureus.

Mode of Action

Coagulase is an enzyme with the ability to coagulate plasma. Staphylococcus aureus forms two types of coagulase. The free coagulase is an extracellular enzyme, the bound coagulase is localized on the surface of the cell wall. Both enzymes are detected in the tube test. With the slide test, only the bound coagulase can be detected.

Method

An overnight broth culture of staph. aureus is incubated with rehydrated EDTA-rabbit plasma. The coagulase test is positive, if more than three quarters of the tube contents forms a coherent clot.

Stability

See expiry date.

Bactident® Coagulase is stable for 5 days in the dissolved (rehydrated) condition at +2°C to +8°C. At -20°C it is stable for up to 30 days.

Storage

Store cool, dry and tightly closed at +2°C to +8°C. Store dissolved plasma at +2°C to +8 °C or deepfrozen at -20°C. Do not refreeze once defrozed.

Safe Disposal

The contents of the tube include bacteria and must be disposed of safely. This can be done by autoclaving or placement in a 5-6% solution of disinfectant for at least 6hours.

Experimental Procedure

- Conduct the coagulase test on 5 typical and/or 5 atypical colonies on BAIRD-PARKER agar (Merck Cat. No. 1.05406.) or 5 suspect colonies from other culture media (CHAPMAN agar, Merck Cat. No. 1.05469., VOGEL-JOHNSON agar, Merck Cat. No. 1.05405., Blood agar (base), Merck Cat. No. 1.10886.).
- Transfer each of the selected colonies with a sterile inoculation loop to separate culture tubes containing brain-heart broth (Merck Cat. No. 1.10493.) and incubate at 37°C for 20-24 hours.
- Dissolve the freeze-dried EDTA-rabbit plasma in 3ml of distilled or demineralized water.
- Pipette 0.3ml of the rehydrated Bactident® Coagulase into a sterile culture tube using a sterile pipette.
- Carefully mix 0.1ml of the brain-heart broth culture 1/2 an inoculation loop of colony material from the CHAPMAN blood or BAIRD-PARKER agar with the 0.3ml of plasma and incubate in a water bath at 37 °C. (Colony material directly from VOGEL-JOHNSON or mannitol-sodium chloride-phenol red agar is not suitable for the test. A brain-heart broth culture is required first.)
- Every hour, check the tube contents for coagulation by gently tipping to the side (not by shaking).
- The coagulase test is positive, if more than three quarters of the tube contents has formed a coherent clot.

If the test is negative after 4-6 hours, continue incubating the tube and make a final assessment after 24hours. For the negative control, prepare a brain-heart broth, but do not inoculate. There must be no sign of clotting.

For the positive control, conduct the test with a coagulase-positive strain of staphylococcus.

negative	no coagulation
1 + positive	a few small separate clots
2 + positive	a few small joined clots
3 + positive	large extensively coagulated clots
4 + positive	complete coagulation, contents do not shift when tube is inverted

Notes

The slide test in which a colony is mixed with rabbit plasma on a microscope slide (clumping factor), only detects the bound coagulase and can at best only service as a screening test. False-positive reactions and autoagglutination can occur.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bactident® Coagulase	1.13306.0001	6 vials each containing 3ml of lyophilized rabbit plasma with EDTA