# THERMONUCLEASE TEST

## Principle

*Staphylococcus aureus* contains a heat-stable thermonuclease and coagulase negative staphylococcus does not. This is a rapid test to differentiate between the two organisms.

## Materials

Toluidine blue-O DNA plate (Q-Lab)
13x100 mm tube with white cap
pasteur pipettes

## Procedure

1. Dispense 2 - 3 mL of blood broth from BacT/Alert bottle showing gram positive cocci in clusters in the direct Gram stain into a sterile capped 13x100 mm tube.
2. Place tube in heating block, $100^0$C for 15 minutes.
3. Let cool to room temperature.
4. Centrifuge at approximately 2500 rpm for 3 minutes.
5. Inoculate a pre-warmed ($35^o$C for 1 hour) toluidine blue-O DNA plate by filling wells (cut well with the end of a pasteur pipette) with 2 drops of the supernatant.
6. Incubate the plate at $35^o$C in the upright position (agar side down).
7. Inspect the plate at, 1 hour, 2 hours and 4 hours and again after overnight incubation if negative at 4 hours.
8. Always run negative and positive control wells with each plate each day.

## Interpretation

Positive: Pink zone of clearing at the edge of the well with a darker blue ring at the outer periphery of the zone; indicates thermonuclease activity

Negative: No zone or a small clear zone around the well

## Quality Control

1. Inoculate 5 day negative patient BacT/Alert bottles with 0.5 mL of a slightly turbid suspension of (a) *S. aureus* (ATCC 25923) and (b) *S. epidermidis* (ATCC 12228) in trypticase soy broth.

2. Incubate the bottles overnight at 36$^{o}$C on the shaker.

3. Remove 3 - 6 mL of the broth-blood from the bottles and process in the same manner as the patient specimens (steps 1 to 4). Always QC new controls before use with patient specimen.

4. Supernatants may be kept refrigerated for up to 1 month for use as controls.

## Reference

1. Rafner, H.B., & Stretton C.W. 1985. Thermonuclease test for same day identification of *S. aureus* in blood cultures. J. Clin. Microbiol. 21:995-996.