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# THERMONUCLEASE AGAR w/ TOLUIDINE BLUE

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## INTENDED USE

Remel Thermonuclease Agar w/ Toluidine Blue is a solid medium recommended for use in qualitative procedures for presumptive identification of *Staphylococcus aureus*.

## SUMMARY AND EXPLANATION

In 1976, Zarzour and Belle reported the usefulness of the thermonuclease test in confirming questionable coagulase-negative *S. aureus* isolates.<sup>1</sup> Shanholtzer and Peterson compared the thermonuclease test with the tube coagulase and clumping factor (slide coagulation) test.<sup>2</sup> They found the thermonuclease test to be as reliable as the tube coagulase and less time consuming to perform. In 1983, Madison and Baselski reported the detection of thermonuclease activity in blood culture samples containing gram-positive cocci.<sup>3</sup> Their study demonstrated 100% correlation with the subsequent identification of the isolate as *S. aureus* by the coagulase test. In 1985, Ratner and Stratton confirmed that the thermonuclease test could be performed directly from a blood culture broth showing gram-positive cocci in clusters on a Gram stain, thus allowing a same-day report of presumptive for *S. aureus*.<sup>4</sup>

## PRINCIPLE

The nuclease produced by *S. aureus* is thermostable, whereas that produced by other organisms is thermolabile.<sup>5-7</sup> Broth cultures incubated for 2 hours followed by boiling for 15 minutes are tested in wells cut into the agar plate. The thermonuclease enzyme produced by *S. aureus* depolymerizes DNA in the area adjacent to the well after incubation of the plate for 2-4 hours. The production of a pink zone of diffusion or halo around the well is due to the metachromatic property of toluidine blue.

## REAGENTS (CLASSICAL FORMULA)\*

Sodium Chloride.....	10.0 g	Deoxyribonucleic Acid.....	0.3 g
TRIS Base.....	6.0 g	Toluidine Blue (0.1 M).....	3.0 ml
Calcium Chloride (0.01 M).....	1.0 g	Agar.....	10.0 g
		Demineralized Water.....	1000.0 ml

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. Inoculate a tube of brain heart infusion broth with several colonies of the isolate to be tested.
2. Incubate the tube for 2 hours at 33-37°C.
3. Boil the broth for 15 minutes. If testing broth from a blood culture, transfer an aliquot of the blood culture broth (including blood cells) to a separate tube and boil the tube for 15 minutes. Allow the broth to cool after boiling.
4. Cut wells into Thermonuclease Agar w/ Toluidine Blue using the blunt end of a Pasteur pipette. Remove the agar plug with the tip of the pipette and discard.
5. Fill the well with 2 drops of the cooled broth.
6. Incubate the plate aerobically for 2-4 hours at 33-37°C. Do not invert the plate.
7. Examine the well for a pink zone or halo at the edge of the well with a darker blue ring at the outer periphery of the zone.

**Pour Tube:** Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above.

## INTERPRETATION OF THE TEST

Positive Test - Pink zone of clearing at the edge of the well with a darker blue ring at the outer periphery of the zone

Negative Test - No zone or a small clear zone (without pink) and no hyper-pigmented peripheral ring

## QUALITY CONTROL

All lot numbers of Thermonuclease Agar w/ Toluidine Blue have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### CONTROL

*Staphylococcus aureus* ATCC® 25923  
*Staphylococcus epidermidis* ATCC® 12228  
*Serratia marcescens* ATCC® 8100

### INCUBATION

Aerobic, 2-4 h @ 33-37°C  
Aerobic, 2-4 h @ 33-37°C  
Aerobic, 2-4 h @ 33-37°C

### RESULTS

Positive  
Negative  
Negative

## LIMITATIONS

1. Some strains of staphylococci are inhibited by Thermonuclease Agar w/ Toluidine Blue.<sup>9</sup>
2. Organisms other than *S. aureus* (e.g., coagulase-negative staphylococci, enterococci, *Bacillus* spp.) may produce thermonuclease.<sup>8</sup>
3. Thermonuclease Agar w/ Toluidine Blue is intended for presumptive identification of *S. aureus*. Additional biochemical testing may be required for definitive identification.<sup>9</sup>
4. The metachromatic color change associated with the production of thermonuclease is stable for 2-4 hours, after which the dye slowly diffuses into the agar and loses its well-demarcated borders.<sup>9</sup>

## BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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