Toluidine Blue DNA Agar, Modified

M613F

Toluidine Blue DNA Agar, Modified is recommended for detection of thermostable deoxyribonuclease activity and establish speciation of *S. aureus* in contaminated foods in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Deoxyriboneuclic acid (DNA)	0.300
Calcium chloride (anhydrous)	0.0011
Sodium chloride	10.000
Toludine blue	0.083
Tris (hydroxy methyl) amino methane	6.100
Agar	10.000
Final pH (at 25°C)	9.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.48 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and continue to boil for 1 to 2 minutes. Sterilization is not necessary. Dispense into sterile Petri plates.

Principle And Interpretation

Toluidine Blue DNA Agar, Modified is recommended for detection of thermostable deoxyribonuclease activity and establish speciation of *S. aureus* in contaminated foods in accordance with FDA BAM, 1998. *Staphylococcus* is a Gram-positive bacteria which includes several species that can cause a wide variety of infections in humans and other animals through infection or the production of toxins. Staphylococcal intoxication worldwide stands out as one of the main food-borne diseases, with a frequency in incidents of microbiological origin second only to salmonellosis(1). Foods that lack hygienic conditions and that are improperly stored can aid in the Staphylococcal growth and toxin production. Since the infection is caused due to toxin, Staphylococcal food poisoning is also called as ‘food intoxication’(2). Toluidine Blue DNA Agar, Modified (M613F) is recommended for detection of thermostable deoxyribonuclease activity and establish speciation of *S. aureus* in contaminated foods in accordance with FDA BAM, 1998(3) with a slight modification in concentration of calcium chloride and toluidine blue. DNA in the medium enables the detection of DNase activity by getting depolymerized and forming a clear zone around the microbial growth. Inclusion of toluidine blue aids in detection of DNase activity by the production of a visible bright rose-pink coloured reaction due to its metachromatic properties.

Total plate count of the suspected sample is carried out using Baird Parker Agar (M043). Speciation of the organism can be confirmed using Toluidine Blue DNA Agar, Modified. Like the coagulase test, this is a highly specific test for the confirmation of the organism. The test is carried out in petriplates or microslides containing the media, prepared by spreading appropriate amount (3ml if slide) of the Toludine Blue DNA Agar, Modified medium. 10 to 12 wells of 1mm diameter are prepared in the plates/slides after solidification. Add 0.01 ml of the broth culture into these wells after heating in a boiling water bath for 15min. Incubate (in a moist chamber) for 4 hrs at 35°C. Development of bright pink halo extending at least 1 mm from periphery of well indicates a positive reaction. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provide the ions and also maintains osmotic equilibrium.

Quality Control

Appearance

Light yellow to light grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Blue coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.65% w/v aqueous solution at 25°C. pH : 9.0±0.2

pH
8.80-9.20

Cultural Response
18 hrs old BHI broth culture is heated in boiling water bath for 15 minutes and studied for thermonuclease activity. 5 mm cut wells are cut in agar plates and is filled with 25-30µl of this culture and incubated at 35-37°C for 4 hrs (or it can also be incubated at 50°C for 2 hrs) and observed for results.

Cultural Response

Organism	**DNase activity**
Cultural Response	
Staphylococcus aureus *ATCC 12600*	positive reaction, pink haloes extending 1mm beyond the well
Staphylococcus epidermidis *ATCC 14990*	negative reaction

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1.Bautista, L., Gaya, P., Medina, M. and Nuñez, M. 1988. Applied and Environmental Microbiology, 54(2): 566-569.
2.Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. 2003. Manual of Clinical Microbiology. 8 ed. Washington, D.C: ASM.
3.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.

Revision : 02 / 2015