



Toluidine Blue DNA Agar

M613I

Intended Use

Recommended for detection of thermostable deoxyribonuclease activity to establish speciation of *S.aureus* in contaminated food. The composition and performance criteria are in accordance with ISO 8870:2006(E) 83:2006(E).

Composition**

ISO 8870:2006(E) 83:2006(E)- Toluidine Blue DNA Agar

Ingredients	g/ L
Deoxyribonucleic acid (DNA)	0.300
Calcium chloride anhydrous	0.110
Sodium chloride	10.000
Toluidine blue O solution	0.093
Tris (hydroxymethyl) amino methane	6.060
Agar	10.000
Final pH (at 25°C)	9.0±0.2

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**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.56 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely and continue to boil for 1 to 2 minutes. Sterilization is not necessary. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The growth of *Staphylococcus aureus* in foods represents a potential public health hazard since many strains of *S.aureus* produce enterotoxins that cause food poisoning if ingested. Numerous outbreaks of staphylococcal intoxication are associated with cheese, stimulating numerous studies on the incidence and behaviour of staphylococci in milk and cheese. Toluidine Blue DNA Agar (M613) is recommended by APHA for detection of the thermostable deoxyribonuclease activity to establish the speciation of *S.aureus* in contaminated foods (1). Toluidine Blue DNA Agar (M613I) is recommended by ISO Committee (2) 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. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provide the ions and also maintains osmotic equilibrium.

Type of specimen

Dairy samples: milk and milk products

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,3).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Pure isolate must be used.
2. Other biochemical tests must be performed.

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

24 hours old BHI broth culture is heated in boiling water bath for 15 minutes and studied for thermonuclease activity. 6.5 mm well are cut in agar plates and is filled with 10µl 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. Incubate for upto 24 hours in case of negative results.

Organism

Staphylococcus aureus subsp.
aureus ATCC 25923 (00034*)

Staphylococcus aureus subsp.
aureus ATCC 6538 (00032*)

Staphylococcus epidermidis
ATCC 14990 (00132*)

DNase activity

positive reaction, pink halos
extending 1mm beyond the well

positive reaction, pink halos
extending 1mm beyond the well

negative reaction

Key : (*) corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
2. Milk and Milk products-Detection of thermonuclease produced by coagulase positive staphylococci- International Organization for Standardization ISO, 8870 :2006 (E), IDF, 83:2006 (E)
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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Disclaimer :

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