Antimicrobial Inhibitor Test Agar pH 6.0, Granulated

GM1631

Antimicrobial Inhibitor Test Agar pH 6.0, granulated is recommended for residual analysis of antimicrobial components in meat and organ samples, using *Bacillus subtilis* (ATCC 6633) as test organism.

Composition**

Ingredients	Gms / Litre
Tryptone	3.500
Meat extract	3.500
Sodium chloride	5.000
Agar	13.000
Final pH (at 25°C)	6.00

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix 1 ml of *Bacillus subtilis* spore suspension per liter of sterile and cooled (50-56°C) medium. Mix thoroughly and pour into sterile Petri plates.

Principle And Interpretation

In addition to washing, treatments with antimicrobial compounds such as chlorine and organic acids are used to sanitize muscle foods. Various concentrations and the degree of effectiveness of the concentrations of these antimicrobial compounds have been reported. Antimicrobial Inhibitor Test Agar pH 6.0 is recommended for residual analysis of antimicrobial components in meat and organ samples, using *Bacillus subtilis* (ATCC 6633) as test organism by agar diffusion procedure and EEC Four-Plate-Test.

Agar Diffusion procedure : Small slices of the meat sample are placed on the seeded Test agar plates and incubated. Antimicrobial components if present in the sample diffuses into the medium and inhibit the growth of test organisms causing growth free inhibition zone. This test is repeated with all the three Antimicrobial Inhibitor Test Agar with three different pH ie. GM1631/M1631(pH 6.0), GM1601/M1601(pH 7.2) and GM1632/M1632 (pH 8.0). Different antibiotic to be analysed such as penicillin, streptomycin and sulphonamide have different pH range for optimal activity for example penicillin G is active optimally at pH 6.0 and streptomycin at pH 8.0 and sulphonamide at pH 7.2.

For EEC Four-Plate-Test method: Molten and cooled antimicrobial inhibitor test agar is seeded with test organisms, *Bacillus subtilis* spores suspension (1), mix well and dispense in petri plates. In one half of the seeded plate, aseptically place two small piece (2-8mm) of meat or organ sample at proper distance and in second half of the plate, place test discs with standard antibiotic to be analyzed, as control. Use disc with 0.01 IU of penicillin-G sodium salt as standard.

Incubate the plates for 18-24 hours at 30°C for *Bacillus subtilis*. After incubation measure the zone of inhibition The inhibition zone between tissue section edge and growth limit of test organism is measured. The zone of at least 2 mm is regarded as positive and less than 2mm (12mm) is considered doubtful. The standard disc should display minimum 6 mm zone of inhibition.

Antimicrobial Inhibitor Test Agar pH 6.0 contains tryptone and meat extract which serve as source for carbon, nitrogen and growth factors for the growth of organisms. Sodium chloride helps to maintain osmotic balance in the medium.

Quality Control

Appearance

Cream to yellow coloured granular medium

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.5% w/v aqueous solution at 25°C. pH : 6.00

Cultural Response

Cultural response and zone of inhibition observed using *B.subtilis* after an incubation at 30°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Inhibition zones with Gentamicin (10mcg)	Inhibition zones with Gentamicin (30mcg)	Inhibition zones with Penicillin (10IU)	Inhibition zones with Streptomycin (10mcg)
Cultural Response							
Bacillus subtilis ATCC 6633	50-100	good-luxuriant	>=70 %	20-28 mm	22-30 mm	36-48 mm	19-27 mm

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. Ferrini, A. M.; Mannoni, V., Aurdi P. Combined plate microbal assay (CPMA). Food additives and Contaminants, 23(1);16-24. 2006

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