Diagnostic Sensitivity Test Agar (D.S.T. Agar)

M502

Diagnostic Sensitivity Test Agar (D.S.T. Agar) is used as an antibiotic sensitivity-testing medium for antibiotic sensitivity testing of fastidious pathogens such as *Neisseria, Streptococcus* and *Haemophilus* species with blood enrichment.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Veal infusion solids	10.000
Dextrose	2.000
Sodium chloride	3.000
Disodium phosphate	2.000
Sodium acetate	1.000
Adenine sulphate	0.010
Guanine hydrochloride	0.010
Uracil	0.010
Xanthine	0.010
Aneurine	0.00002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.04 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. For blood agar, cool the base to 50°C and add 7% v/v sterile defibrinated horse blood aseptically. Mix well with gentle rotation and pour into sterile Petri plates.

Principle And Interpretation

Diagnostic Sensitivity Test Agar is recommended for diagnostic as well as testing susceptibility of organisms to antibiotics and chemotherapeutic agents such as Sulponamides. The latter produce well defined zones due to the absence of interfering substances.

The medium is nutritionally rich due to presence of amino acid bases and glucose. The salts present, helps in avoiding sudden pH shifts due to acid production, which might affect the susceptibility test and haemolytic reactions (1) and the MIC values of pH susceptible antimicrobials (2). Aneurine acts as vitamin source which improves the growth of several organisms especially Staphylococci. The agar used in the formulation has been specially processed to allow unimpeded diffusion of antimicrobials from discs (3). Addition of the bases like adenine, guanine, uracil and xanthine improve the antibiotic testing performance of the medium.

The reactive levels of thymidine and thymine must be sufficiently reduced to avoid antagonism of trimethoprim and sulphonamides which is an essential requirement for satisfactory antimicrobial susceptibility media. The requirement is achieved by addition of lysed horse blood to Diagnostic Sensitivity Testing medium. The level of thymidine is further reduced due to the action of thymidine phosphorylase, released from lysed horse erythrocytes (4). Thymidine-dependant organisms will not grow in absence of thymidine or will grow poorly in media containing reduced levels (5).

For less demanding organisms like Micrococci,! Salmonella, Shigella @, coliform bacteria and *Proteus* species, this medium can be used without blood. For fastidious organisms like *Haemophilus influenzae* , *Neisseria meningitides @, alpha and beta haemolytic Streptococci blood enrichment is necessary.*

Antibiotic susceptibility test is performed as follows: Suspension of test organisms is spread on the surface of the medium. Sensitivity discs (3) are equally spaced on the seeded medium surface and incubated at 37°C for 18 hours. The zones of

inhibition obtained are recorded. This medium has reduced thymidine activity and this will affect its performance as a primary isolation medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium : Light amber coloured, clear to slightly opalescent gel forms. After addition of 7%w/v sterile defibrinated blood : Cherry red coloured, opaque gel forms in Petri plates.

Reaction
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M502: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=70%
Micrococcus luteus ATCC 10240	50-100	luxuriant	>=70%
Neisseria meningitidis ATCC 13090	50-100	luxuriant (with the addition of blood)	>=70%
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=70%
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=70%
Shigella flexneri ATCC 12022	50-100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Streptococcus pneumoniae ATCC 6305	50-100	luxuriant(with the addition of blood)	>=70%
Streptococcus pyogenes ATCC 19615	50-100	Luxuriant (with the addition of blood)	>=70%

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. Expert Committee on antibiotics, 1961, World Health Organisation Technical Report Series No. 210, WHO, Geneva.
2. Bechtle R. M. and Schere G. H., 1958, Antibiotics and Chemotherapy, 8(12): 599.
3. Marshall J. H. and Kelsey J. C., 1960, J. Hyg. Camb., 58 : 367.
4. Ferone R., Bushby S. R. M., Burchall J. J., Moore W. D., and Smith D., 1975, Antimicrobial Agents Chemotherap., 7: 91-98

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