PRODUCT SPECIFICATION SHEET

Diagnostic Sensitivity Test Agar (D.S.T. Agar) (DM695)

Intended Use

Diagnostic Sensitivity Test Agar (D.S.T. Agar) (DM695) is recommended for antibiotic sensitivity testing of fastidious pathogens such as *Neisseria*, *Streptococcus* and *Haemophillus* species with blood enrichment.

Product Summary and Explanation

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.

Principles of the Procedure

Diagnostic Sensitivity Test Agar is nutritionally rich as it comprises 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] Bases like adenine, guanine, uracil and xanthine are added to 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. Addition of lysed horse blood to Diagnostic Sensitivity Testing medium helps to achieve this requirement. Thymidine phosphorylase, released from lysed horse erythrocytes further reduces the level of thymidine.[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.*

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	10.00
Veal infusion solids	10.00
Dextrose	2.00
Sodium chloride	3.00
Disodium phosphate	2.00
Sodium acetate	1.00
Adenine sulphate	0.01
Guanine hydrochloride	0.01
Uracil	0.01
Xanthine	0.01
Aneurine	0.00002
Agar	15.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

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Directions

1. Suspend 43.04 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. For blood agar, cool the base to 50°C and add 7% v/v sterile defibrinated horse blood aseptically.
5. Mix well with gentle rotation and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
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 of 4.3% Solution	pH : 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel.

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

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	>=70%
2.	*Enterococcus faecalis ATCC 29212*	50-100	good-luxuriant	>=70%
3.	*Micrococcus luteus ATCC 10240*	50-100	good-luxuriant	>=70%
4.	*Neisseria meningitidis ATCC 13090*	50-100	good-luxuriant (with the addition of blood)	>=70%
5.	*Proteus mirabilis ATCC 25933*	50-100	good-luxuriant	>=70%
6.	*Salmonella Typhi ATCC 6539*	50-100	good-luxuriant	>=70%
7.	*Shigella flexneri ATCC 12022*	50-100	good-luxuriant	>=70%
8.	*Staphylococcus aureus ATCC 25923*	50-100	good-luxuriant	>=70%
9.	*Streptococcus pneumonia ATCC 6305*	50-100	good-luxuriant (with the addition of blood)	>=70%
10.	*Streptococcus pyogenes ATCC 19615*	50-100	good-luxuriant (with the addition of blood)	>=70%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Antibiotic susceptibility test is performed as follows:
   a) Suspension of test organisms is spread on the surface of the medium.
   b) Sensitivity discs are equally spaced on the seeded medium surface and incubated at 37°C for 18 hours.
2. Refer to appropriate references for standard test procedures.

Results

The zones of inhibition obtained are recorded. Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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Limitations of the Procedure

1. This medium has reduced thymidine activity and this will affect its performance as a primary isolation medium.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name Diagnostic Sensitivity Test Agar (D.S.T. Agar)
Product Code : DM695
Available Pack sizes : 100gm/ 500gm

References

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
5. Stokes E. J. and Ridgway G. L. (1980) 'Clinical Bacteriology' 5th Edn. Arnold. London, 54.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

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	Checked By	Approved By
Microbiologist	**Head Quality Control**	**Head Quality Assurance**