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

M502

Intended Use:

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 from clinical samples.

Composition**

Ingredients	g / L
Proteose peptone	10.000
HMV infusion solids #	10.000
Dextrose (Glucose)	2.000
Sodium chloride	3.000
Disodium hydrogen 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
Equivalent to Veal infusion solids

Directions

Suspend 43.04 grams in 1000 ml purified / 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 45-50°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 Sulfonamides. 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 sulfonamides 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 (1). Thymidine-dependent organisms will not grow in absence of thymidine or will grow poorly in media containing reduced levels (4).

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 (5) 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.

Type of specimen

Clinical samples: Isolated samples from Blood, urine, respiratory samples, and other clinical material.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. 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).
2. Some fastidious organisms may not grow.
3. Inoculum density affects inhibition zone. Heavy inoculum may result in smaller zones while scanty growth may result in enlarged zones.

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.

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

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

Organism	Inoculum (CFU)	Growth	Recovery
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=70%
Micrococcus luteus ATCC 10240	50-100	luxuriant (with the addition of blood)	>=70%
Salmonella Typhi ATCC 6539	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%
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	>=70%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Use before expiry date on the label. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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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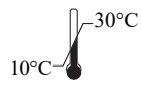
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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