



Soyabean Casein Digest Agar (Tryptone Soya Agar) (Casein Soyabean Digest Agar)

M290

Intended use

For cultivation of a wide variety of microorganisms from clinical and non-clinical samples and for sterility testing in pharmaceutical procedures.

Composition**

Ingredients	g/ L
Tryptone #	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of casein

Directions

Suspend 40.00 gram 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. If desired, aseptically add 5% v/v defibrinated blood in previously cooled medium to 45-50°C for cultivation. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Soyabean Casein Digest Agar is a widely used medium, which supports the growth of wide variety of organisms even that of fastidious ones such as *Neisseria*, *Listeria*, and *Brucella* etc. The medium with addition of blood provides perfectly defined haemolysis zones, while preventing the lysis of erythrocytes due to its sodium chloride content. It has been frequently used in the health industry to produce antigens, toxins etc. It's simple and inhibitor-free composition makes it suitable for the detection of antimicrobial agents in the food and other products.

Tryptone Soya Agar is recommended by various pharmacopoeias as sterility testing medium (1,2). Tryptone Soya Agar conforms as per USP (1) and is used in microbial limit test and antimicrobial preservative - effective test. Gunn et al (3) used this medium for the growth of fastidious organisms and study of haemolytic reaction after addition of 5%v/v blood. The combination of tryptone and soya peptone makes this media nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Sodium chloride maintains the osmotic balance. Soyabean Casein Digest Agar does not contain X and V growth factors. It can be conveniently used in determining the requirements of these growth factors by isolates of *Haemophilus* by the addition of X-factor (DD020), V-factor (DD021), and X+V factor discs (DD022) factor to inoculated TSA plates (4).

Type of specimen

Pharmaceutical samples, Clinical samples- urine, faeces, abscess etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For pharmaceutical samples follow appropriate techniques for sample collection, handling and processing as per pharmacopoeias (1,2).

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

Warning and Precautions

In Vitro diagnostic Use. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological tests must be carried out for confirmation.

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 yellow coloured clear to slightly opalescent gel. After addition of 5-7%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

Reaction

pH of 4.0% w/v aqueous solution at 25°C .

pH

7.10-7.50

Cultural response

Productivity : Cultural characteristics was observed after an incubation for Bacterial at 30-35°C 18-24 hours and for Fungal at 30-35°C <=5 days. Growth of fungal cultures is also observed at 20-25°C for <=5 days

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Observed Lot value (CFU) w/blood	Recovery w/ blood	Haemolysis
Productivity						
** <i>Bacillus spizizenii</i> ATCC 6633 (00003)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034)*	50 -100	35 -100	>=70 %	35 -100	>=70%	beta
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	beta
<i>Escherichia coli</i> ATCC 25922 (00013)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
<i>Escherichia coli</i> ATCC 8739 (00012)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
<i>Escherichia coli</i> ATCC 11775 (00090)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
<i>Escherichia coli</i> NCTC 13167 (00179)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
^ <i>Pseudomonas paraaeruginosa</i> ATCC 9027 (00026)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Pseudomonas aeruginosa</i> ATCC 10145 (00024)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Salmonella</i> Typhimurium ATCC 14028 (00031)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Salmonella</i> Abony NCTC 6017 (00029)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
§ <i>Kokuria rhizophila</i> ATCC 9341	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

<i>Streptococcus pneumoniae</i> ATCC 6305	50 -100	35 -100	>=70 %	35 -100	>=70 %	alpha
<i>Enterococcus faecalis</i> ATCC 29212 (00087)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Clostridium sporogenes</i> ATCC 19404 (00008)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Candida albicans</i> ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Candida albicans</i> ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
# <i>Aspergillus brasiliensis</i> ATCC 16404 (00053)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
After an incubation at 20-25°C for <=5 days						
<i>Candida albicans</i> ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
<i>Candida albicans</i> ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
# <i>Aspergillus brasiliensis</i> ATCC 16404 (00053)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

Key : (*)- Corresponding WDCM numbers,

(**) Formerly known as *Bacillus subtilis* subsp. *spizizenii* , (^) Formerly known as *Pseudomonas aeruginosa*,

(\$) Formerly known as *Micrococcus luteus* ,

(*) Formerly known as *Aspergillus niger*

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

- 1.The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
- 2.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India
- 3.Gunn B. A., Ohashi D K., Gaydos C. A., Holt E. S., 1977, J. Clin. Microbiol., 5(6) : 650.
- 4.Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey and Scotts Diagnostic Microbiology, 10th Ed., Mosby Inc. St. Louis, Mo
- 5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
- 6.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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