Technical Information

Tinsdale Agar Base

Product Code: DM 1314

Application: Tinsdale Agar Base with supplement is used for selective isolation and differentiation of *Corynebacterium diphtheriae.*

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Sodium chloride	5.000
L-Cystine	0.240
Sodium thiosulphate	0.430
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The Corynebacteria are gram-positive, non-sporulating, non-motile rods. They are often club-shaped and frequently banded or beaded with irregularly stained granules. These bacteria are generally aerobic or facultative, but microaerophilic species do occur. Corynebacterium diphtheriae produces a powerful exotoxin that causes diphtheria in humans. In nature, *C. diphtheriae* is isolated from nasopharyngeal area of infected persons or healthy carriers.

There are three biotypes of *C. diphtheriae* normaly mitis, intermedius and gravis [6]. The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea [2]. Tinsdale Agar Base Medium was devised by Tinsdale [1] for the isolation and differentiation of *C. diphtheriae* from diphtheroids which was modified by Billings [2], to improve the recovery and differential qualities of *C. diphtheriae.* Moore and Parsons [3] confirmed the halo formation a characteristic property of *C. diphtheriae* with the exception of *C. ulcerans,* which forms colony with similar features as *C. diphtheriae*.

Peptic digest of animal tissue provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H_2S indicator system. Potassium tellurite from the supplement inhibits the growth of all gram-negative bacteria including the upper respiratory tract normal flora.

C. diphtheriae forms grayish black colonies surrounded by a dark brown halo where as diphtheroids commonly found in the upper respiratory fail to form such colonies. Dark brown halo around the colony is due to H_2S production from cystine combining with the tellurite salt. Moore and Parsons [3] found Tinsdale Medium an ideal medium for the routine cultivation and isolation of *C. diphtheriae*. They also confirmed the stability of halo formation on clear medium and its specificity for *C. diphtheriae* and *C. ulcerans*. *C. ulcerans* found in nasopharynx form colonies same as *C. diphtheriae* and require further biochemical confirmation [4].

Plates should not be incubated in 5-10% CO_2 as it retards the development of characteristic halos [5]. Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of C. diphtheriae [6]. *C. ulcerans, C. pseudotuberculosis* and (rarely) Staphylococcus species may produce a characteristic halo on Tinsdale Agar [6]. Several organisms may show slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period of 48 hours [6].

Methodology

Suspend 40.67 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add Diphtheria Virulence Supplement (MS2073, Part A and Part B). Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH range 7.20-7.60

Cultural Response/Characteristics
DM 1314: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Diptheria Virulence Supplement (MS2073, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Corynebacterium diphtheriae type gravis	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type interme dius	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type mitis	50-100	good-luxuriant	>=50%	brown-black with halo
Klebsiella pneumoniae ATCC 13883	>=10^3	inhibited	0%	
Streptococcus pyogenes ATCC 19615	50-100	good	40-50%	black pin point, without halo

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C
6. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.

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