PRODUCT SPECIFICATION SHEET

Tinsdale Agar Base (DM267)

Intended Use
Tinsdale Agar Base is used for the selective isolation and differentiation of *Corynebacterium diphtheriae*.

Product Summary and Explanation
Tinsdale Agar Base, supplemented with Tinsdale Enrichment, is employed in the cultural diagnosis of Diphtheria. Diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin[(1)]is caused by toxigenic strains of *Corynebacterium diphtheriae*. The three biotypes are mitis, intermedius and gravis.[(1)] The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.[(2)] Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.[(2)] Tinsdale[(3)]developed a serum-cystine-thiosulfate-tellurite agar medium for the primary isolation and differentiation of *C. diphtheriae*. This formulation distinguished between *C. diphtheriae* and diphtheroids which exhibited similar characteristics. The differential principle is based on the capacity of *C. diphtheriae* to produce a brown or black halo around the colonies. Billings[(4)] simplified Tinsdale Basal Medium by using Meat Peptone as a nutrient source. This modification improved the differential qualities and recovery of *C. diphtheriae*. Moore and Parsons[(5)] confirmed the halo formation of *C. diphtheriae* with one exception; *C. ulcerans* occasionally produced colonies similar to *C. diphtheriae* and required biochemical identification. 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* occurs in nasopharyngeal area of infected persons or healthy carriers.

Principles of the Procedure
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 all gram-negative bacteria and most of the upper respiratory tract normal flora. *C. diphtheriae* forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H_2S production from cystine combining with the tellurite salt.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	20.00
Sodium chloride	5.00
L-Cystine	0.24
Sodium thiosulphate	0.43
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.

Directions
1. Suspend 40.67 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.

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3. Autoclave at 121°C , 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.06% 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 - 48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Corynebacterium diphtheriae type gravis*	50-100	Good-luxuriant	>=50%
2.	*Corynebacterium diphtheriae type intermedius*	50-100	Good-luxuriant	>=50%
3.	*Corynebacterium diphtheriae type mitis*	50-100	Good-luxuriant	>=50%
4.	*Klebsiella pneumoniae ATCC 13883*	>=10^3	Inhibited	0 %
5.	*Streptococcus pyogenes ATCC 19615*	50-100	Good	40-50%

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

Test Procedure

1. Inoculate the medium to obtain well separated colonies.
2. Stab deep into the agar at intervals in order to initiate browning at an early stage (10±12 hours incubation).
3. Plates are incubated at 35-37°C and examined after 24 hours and 48 hours incubation.
4. Growth of C. diphtheriae may be inhibited if Tinsdale Agar is incubated in carbon dioxide-enriched air e.g. in a CO_2 incubator.
5. Browning may be regarded as presumptive evidence of the presence of Corynebacterium diphtheriae although 48 hours incubation may be necessary for the recognition of characteristic colonies.

Results

The appearance of brown-black colored colonies surrounded by brown-black halos is presumptive evidence for *C. diphtheria.*[1]

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. Further tests must be carried out on colonies suspected as *C. diphtheriae*, including cell morphology after sub-culture to Loeffler's Medium and examination for toxin production.
2. Do not incubate Tinsdale's Agar plates in enhanced CO_2 atmosphere (5-10% v/v).

Packaging

Product Name : Tinsdale Agar Base
Product Code : DM267
Available Pack sizes : 100gm / 500gm

References

1. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
2. Funke and Bernard. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Tinsdale. 1947. J. Pathol. Bacteriol. *59*:461.
4. Billings. 1956. An investigation of Tinsdale Tellurite medium: its usefulness and mechanisms of haloformation. M.S. thesis. University of Michigan, Ann Arbor, Mich.
5. Moore and Parsons. 1958. J. Infect. Dis. *102*:88.

Further Information

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