Tinsdale Agar Base Tinsdale Enrichment Desiccated

Intended Use

Tinsdale Agar Base is used with Tinsdale Enrichment Desiccated in isolating and differentiating *Corynebacterium diphtheriae*.

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

Tinsdale³ 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.

Billings⁴ simplified Tinsdale Basal Medium by using Proteose Peptone No. 3 as a nutrient source. This modification improved the differential qualities and recovery of *C. diphtheriae*. Moore and

User Quality Control

<i>Identity Specifications</i> Difco™ Tinsdale Agar Base					
Dehydrated Appearance:	Light beige, free flowing, homogeneous.				
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent.				
Prepared Appearance:	Light to medium amber, slightly opalescent to opalescent.				
Reaction of 4.5% Solution at 25°C:	pH 7.4 ± 0.2				

Difco[™] Tinsdale Enrichment Desiccated

Desiccated Appearance: Light to dark tan cake; variations may occur. Solution: Soluble in purified water. Solution is light to dark amber, clear to opalescent, may have a slight precipitate.

Cultural Response Difco™ Tinsdale Agar Base with Tinsdale Enrichment Desiccated

Prepare the medium per label directions. Inoculate to obtain discrete colonies and stab several times using an inoculating needle; incubate at $35 \pm 2^{\circ}$ C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Corynebacterium diphtheriae biotype gravis	8028	10 ² -10 ³	Good	Brown with halos
<i>Corynebacterium diphtheriae</i> biotype mitis	8024	10 ² -10 ³	Good	Brown with halos
Klebsiella pneumoniae	13883	10 ² -10 ³	Marked to complete inhibition	-
Streptococcus pyogenes	19615	10 ² -10 ³	Poor to fair	Brown to black without halos

Parsons⁵ confirmed the halo formation of *C. diphtheriae* with one exception; *C. ulcerans* occasionally produced colonies similar to *C. diphtheriae* and required biochemical identification.

Principles of the Procedure

Peptone provides the nitrogen, vitamins, carbon and amino acids in Tinsdale Agar Base. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Tinsdale Enrichment Desiccated contains bovine serum and horse serum, which provide essential growth factors. L-cystine and sodium thiosulfate provide sulfur for H_2S production. Potassium tellurite is a selective agent. The formation of black to brown halos surrounding the colony results from the reduction of potassium tellurite by H_2S to metallic tellurite.

Stabbing the medium with an inoculating needle accentuates darkening of the medium by *C. diphtheriae*.

Formulae

Difco[™] Tinsdale Agar Base

Approximate Formula* Per Liter		
Proteose Peptone No. 3		g
Sodium Chloride	5.0	g
Agar		g





Difco[™] Tinsdale Enrichment Desiccated

Approximate Formula* Per Liter

Bovine Serum	mL
Horse Serum	mL
L-Cystine	g
Potassium Tellurite	g
Sodium Thiosulfate	g
*Adjusted and/or supplemented as required to meet performance criteria	

Directions for Preparation from Dehydrated Product Difco[™] Tinsdale Agar Base

- 1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. Dispense 100 mL amounts into flasks.
- 4. Autoclave at 121°C for 15 minutes.
- 5. Aseptically add 15 mL rehydrated Tinsdale Enrichment to each 100 mL at 50-55°C. Mix well.
- 6. Test samples of the finished product for performance using stable, typical control cultures.

Difco[™] Tinsdale Enrichment Desiccated

- 1. Rehydrate with 15 mL sterile purified water.
- 2. Rotate in an end-over-end motion to dissolve completely.

Procedure

- 1. For a complete discussion on the collection, isolation and identification of C. diphtheriae and other Corynebacterium species, refer to the appropriate procedures outlined in the references.1,2,6
- 2. Inoculate plates with the test organisms in a manner to obtain discrete colonies and stab the medium several times with an inoculating needle.
- 3. Definitive identification of a strain of C. *diphtheriae* as a true pathogen requires demonstration of toxin production.6

Expected Results

The appearance of brown-black colored colonies surrounded by brown-black halos is presumptive evidence for C. diphtheriae.¹

Limitations of the Procedure

- 1. Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of C. diphtheriae.¹
- 2. C. ulcerans, C. pseudotuberculosis and (rarely) Staphylococcus species may produce a characteristic halo on Tinsdale Agar.1

- 3. Do not read Tinsdale Agar early because several organisms may exhibit slight browning on this medium in 18 hours.¹
- 4. Incubation in 5-10% CO₂ retards the development of halos on Tinsdale Agar.¹
- 5. On media containing tellurite, diphtheria bacilli are shorter and stain more uniformly; however, granules are less readily observed than when grown on Loeffler's medium.7
- 6. Further biochemical tests may be necessary to distinguish between C. diphtheriae and C. ulcerans due to similar reactions on this medium.

References

- Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- 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. Tinsdale. 1947. J. Pathol. Bacteriol. 59:461. Billings. 1956. An investigation of Tinsdale Tellurite medium: its usefulness and mechanisms of halo-

- formation. M.S. thesis. University of Michigan, Ann Arbor, Mich. Moore and Parsons. 1958. J. Infect. Dis. 102:88. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
- Bailey and Scott. 1966. Diagnostic microbiology, 2nd ed. The C. V. Mosby Company, St. Louis, Mo.

Availability Difco[™] Tinsdale Agar Base

Cat. No. 278610 Dehydrated - 500 g

Difco[™] Tinsdale Enrichment Desiccated

Cat. No. 234210 Tube - 6 × 15 mL* *Store at 2-8°C.

