TINSDALE AGAR BASE and ENRICHMENT

INTENDED USE
Remel Tinsdale Medium (agar base and enrichment combined) is a solid medium recommended for use in qualitative procedures for the differentiation of Corynebacterium species.

SUMMARY AND EXPLANATION
In 1947, Tinsdale developed a serum-cystine-sodium thiosulfate-tellurite medium for the primary isolation and differentiation of Corynebacterium diphtheriae. This differentiation was based on the ability of C. diphtheriae to produce black colonies surrounded by a brown-black halo after 48 hours incubation at 37°C. Diphtheroids and other microorganisms of the respiratory tract do not have this ability. Moore and Parsons further investigated Tinsdale medium and demonstrated that the distinct halo was specific and stable, appearing only around colonies of C. diphtheriae and Corynebacterium ulcerans.

PRINCIPLE
The peptone in this medium provides nitrogen and carbon compounds, amino acids, and trace minerals which promote the growth of microorganisms. Sodium chloride maintains osmotic equilibrium. Potassium tellurite is inhibitory to a variety of gram-positive and gram-negative bacteria but corynebacteria produce gray-black colonies when cultivated in its presence. The halos produced by C. diphtheriae and C. ulcerans arise from the interaction of the potassium tellurite with the hydrogen sulfide produced from L-cysteine. Sodium thiosulfate is the reducing agent in this medium, which hastens the appearance of halos. Serum (rabbit) contains growth factors required by Corynebacterium species.

REAGENTS (CLASSICAL FORMULAE)*
Tinsdale Agar Base:
- Peptone .................................................. 20.0 g
- Sodium Chloride ...................................... 5.0 g
- Agar .................................................. 20.0 g
- Demineralized Water .......................... 1000.0 ml
- pH 7.4 +/- 0.2 @ 25°C

Tinsdale Enrichment:
- Rabbit Serum ........................................... 100.0 ml
- Potassium Tellurite 1% .......................... 30.0 ml
- Sodium Thiosulfate 2.5% ..................... 17.0 ml

Rabbit Serum...................................................................... 100.0 ml
L-Cysteine Hydrochloride 1%................................................17.5 ml
Sodium Thiosulfate 2.5% ......................................................17.0 ml

*Adjusted as required to meet performance standards.

PROCEDURE
1. Melt Tinsdale Agar Base (REF R09897) in a boiling water bath and cool to 45-50°C.
2. Add 3.3 ml of sterile demineralized water to Tinsdale Enrichment (REF R45045) and swirl to mix.
3. Add the enrichment to the cooled base, invert the tube to mix, and dispense into a sterile Petri dish.
4. Incubate the specimen as soon as possible after it is received in the laboratory.
5. Incubate aerobically at 35-37°C for 24-48 hours.
6. Observe for growth and tellurite reduction.
7. Colorless colonies without halos

INTERPRETATION OF THE TEST
Positive Test -  Gray-black colonies surrounded by intense brown halos
Negative Test -  Colorless colonies without halos

QUALITY CONTROL
All lot numbers of Tinsdale Agar Base and Tinsdale Enrichment have been tested using the following quality control organism and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL
Corynebacterium diphtheriae ATCC® 13812

INCUBATION
Aerobic, 48 h @ 35-37°C

RESULTS
Gray-black colonies with brown halos

LIMITATIONS
1. Any browning of the medium is presumptive evidence of the presence of C. diphtheriae, although 48 hours incubation may be necessary for recognition of characteristic colonies.
2. Dark brown halos around colonies are usually produced only by C. diphtheriae and C. ulcerans; however, rare strains of diphtheroids, staphylococci, streptococci, and other bacteria may produce halos around dark colonies.
3. Incubation in a 5-10% CO₂ atmosphere retards the development of characteristic halos.

BIBLIOGRAPHY

Refer to the front of Remel Technical Manual of Microbiological Media for General Information regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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