# Tinsdale Agar Base

M314

## Intended Use:

Recommended for selective isolation and differentiation of *Corynebacterium diphtheriae.*

## Composition**

| Ingredients | g / L |
|---|---|
| Peptone | 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

## Directions

Suspend 40.67 grams 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. Cool to 45-50°C and aseptically add Tinsdale Selective Supplement (FD073, Part A and Part B). Mix well and pour into sterile Petri plates.

## Principle And 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* occurs in nasopharyngeal area of infected persons or healthy carriers.

The three biotypes of *C.diphtheriae* are mitis, intermedius and gravis (1). The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea (2). Tinsdale Agar Base Medium was developed by Tinsdale (3,4) for the selective isolation and differentiation of *C.diphtheriae* from diphtheroids. This medium was modified by Billings (2), which improved the recovery and differential qualities of *C.diphtheriae.* The present medium is according to the modified Billings Medium. Moore and Parsons (3) confirmed the halo formation as a characteristic property of *C.diphtheria* with the exception of *C.ulcerans,* which forms colony with similar features as *C.diphtheriae.*

Peptone 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 H2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as 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 (5).

Do not incubate the plates in 5-10% CO2 as it retards the development of characteristic halos (6). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C.diphtheriae* (1). *C.ulcerans* , *C.pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (1).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (1).

## Type of specimen

Clinical samples - Throat swab

## Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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

## Warning and Precautions :

In Vitro diagnostic Use only. 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. Do not incubate the plates in 5-10% $CO_2$ as it retards the development of characteristic halos (6).

## 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**

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**

7.20-7.60

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Tinsdale Selective Supplement (FD073, 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 intermedius* | 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 (00097*) | >=$10^4$ | inhibited | 0 % | |
| *Streptococcus pyogenes* ATCC 19615 | 50-100 | good | 40-50% | black pin point, without halo |

Key : *Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (7,8).

## Reference

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5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. 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
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8. 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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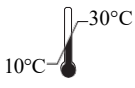


**Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

**CE Marking**

**Do not use if package is damaged**

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