# TM 450 – TINSDALE AGAR BASE

## INTENDED USE

For selective isolation and differentiation of *Corynebacterium diphtheria*.

## PRODUCT SUMMARY AND EXPLANATION

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

*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. Moore and Parsons 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.

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

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **Peptic digest of animal tissue** | 20.000 |
| **Sodium chloride** | 5.000 |
| **L-Cystine** | 0.240 |
| **Sodium thiosulphate** | 0.430 |
| **Agar** | 15.000 |

## PRINCIPLE

Peptic digest of animal tissue provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

## INSTRUCTION FOR USE

- Dissolve 40.67 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 50°C and aseptically add Diphtheria Virulence Supplement. Mix well and pour into sterile Petri plates.

## QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.
**Appearance of prepared medium** : Light amber coloured clear to slightly opalescent gel forms in Petri plates.
**pH (at 25°C)** : 7.4±0.2

## INTERPRETATION

Cultural characteristics observed after incubation with added Diptheria Virulence supplement.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Colony characteristics | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|---|---|
| *Corynebacterium diphtheriae type gravis* | 8028 | 50-100 | Good-luxuriant | >=50% | Brown-black with halo | 35-37°C | 40-48 Hours |
| *Corynebacterium diphtheriae type mitis* | 8024 | 50-100 | Good-luxuriant | >=50% | Brown-black with halo | 35-37°C | 40-48 Hours |
| *Klebsiella pneumoniae* | 13883 | >=$10^3$ | Inhibited | 0 % | - | 35-37°C | 40-48 Hours |
| *Streptococcus pyogenes* | 19615 | 50-100 | Good | 40-50% | Black pin point, without halo | 35-37°C | 40-48 Hours |

## PACKAGING:

In pack size of 500 gm bottles.

## STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

1. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
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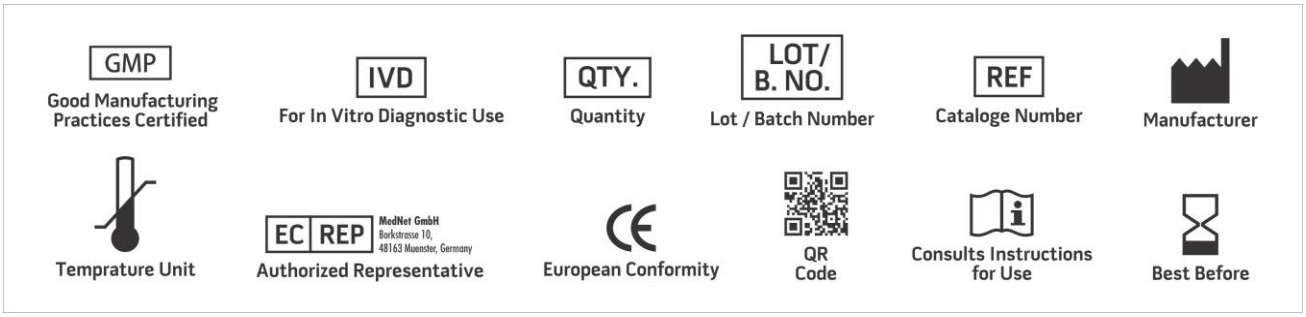
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 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.




**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

**Revision: 08 Nov., 2019**