

# TM 522 - HOYLE MEDIUM BASE

#### **INTENDED USE**

For differentiation and isolation of Corynebacterium diphtheria.

### PRODUCT SUMMARY AND EXPLANATION

The most common disease caused by Corynebacterium diphtheriae is diphtheria, an acute communicable disease manifested by both local infection of the upper respiratory tract and the systemic effects of the toxin, which are most notable in the heart and peripheral nerves. Hoyle Medium Base, formulated by Hoyle, is the modification of the original formulation of Neill, for the isolation and differentiation of C. diphtheriae. This medium is not inhibitory to some mitis types of *Corynebacterium*, as the original formulation.

Hoyle's Medium is a highly selective medium and should be used in conjunction with a non-selective media such as Loeffler Serum Medium and Blood Agar Base with 10% horse blood. C.diphtheriae are usually present in small numbers permitting the formation of well isolated colonies. So, inoculation is done by directly rubbing the swab over the entire surface of the medium. Incubation should be carried out till 72 hours if the results are negative. To study the morphology, gentian violet staining is done. To demonstrate the characteristic morphology and staining reactions of C. diphtheriae by Neissers or Alberts method, it is advisable to use colonies from Loeffler Medium. The toxigenicity of *C. diphtheriae* strains can be determined by Eleks method.

### **COMPOSITION**

| Ingredients     | Gms / Ltr |
|-----------------|-----------|
| Peptone         | 10.000    |
| Beef extract    | 10.000    |
| Sodium chloride | 5.000     |
| Agar            | 15.000    |

# **PRINCIPLE**

Peptone and Beef extract supply carbon, nitrogen substances, amino acids, vitamins and other essential growth nutrients. Potassium tellurite is a selective agent, which inhibits most of the normal flora of the upper respiratory tract except Corynebacterium.

## **INSTRUCTION FOR USE**

- Dissolve 40.0 grams in 940 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C and aseptically add 50 ml of laked blood and 10 ml of 3.5% Potassium Tellurite Solution.
- 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 : Basal Medium: Amber coloured, clear to slightly opalescent gel. After Addition

of blood & Tellurite: Brownish red coloured opaque gel forms in Petri plates.

pH (at 25°C) : 7.8±0.2

### INTERPRETATION

Cultural characteristics observed with added 50 ml of laked blood and tellurite solution, after an incubation.













| Microorganism                          | ATCC  | Inoculum<br>(CFU/ml) | Growth             | Recovery | Colony<br>characteristics    | Incubation<br>Temperature | Incubation<br>Period |
|--|-------|----------------------|--------------------|----------|------------------------------|---------------------------|----------------------|
| Bacillus subtilis<br>subsp. spizizenii | 6633  | >=10 <sup>3</sup>    | Inhibited          | 0%       | -                            | 35 - 37°C                 | 18-24<br>Hours       |
| C. diphtheriae type intermedius        | 14779 | 50-100               | Good-<br>luxuriant | >=50%    | Grey colonies<br>with darker | 35 - 37°C                 | 18-24<br>Hours       |
| Escherichia coli                       | 25922 | >=10 <sup>3</sup>    | Inhibited          | 0%       | -                            | 35 - 37°C                 | 18-24<br>Hours       |
| Enterococcus<br>faecalis               | 29212 | 50-100               | Good-<br>luxuriant | >=50%    | Black minute colonies        | 35 - 37°C                 | 18-24<br>Hours       |

#### **PACKAGING:**

In pack size of 100 gm and 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**

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- 2. Hoyle I., 1941, Lancet., 1:175.
- 3. Isenberg, H.D. Clinical Microbiology Procedures Handbook  $2^{\mbox{nd}}$  Edition.
- 4. 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.
- 5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1,

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- 6. Williams and Wilkins, Baltimore.
- 7. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., 8th Ed., American Society for Microbiology, Washington, D.C. (Ed.), 2003, Manual of Clinical Microbiology.





































**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only

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