# Levinthals Medium Base

**M472**

Levinthals Medium Base with the addition of blood is used for cultivation of *Haemophilus* species.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptic digest of animal tissue | 10.000 |
| Beef extract | 10.000 |
| Sodium chloride | 5.000 |
| Agar | 20.000 |
| Final pH ( at 25°C) | 7.6±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 45 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 100 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and add 5 ml sterile rabbit or human blood to 100 ml medium. Heat the mixture in boiling water bath. Allow the deposits to settle and dispense clear supernatant.

## Principle And Interpretation

The genus *Haemophilus* includes a number of species that cause a wide variety of infections but share a common morphology and a requirement for blood-derived factors during growth that has given the genus its name. *Haemophilus influenzae* , the major pathogen, is by far the most virulent organism in this group, commonly causing bloodstream invasion and meningitis in children younger than 2 years. Other *Haemophilus* species cause disease less frequently. The *Haemophilus* genus represents a large group of gram-negative rods that grow on blood agar. The blood provides two factors, which many *Haemophilus* species require for growth: factor-X and factor-V (1). Levinthals Medium is used for the cultivation of *Haemophilus* species. *Haemophilus* species require haemoglobin for their growth in the culture medium.

Whole blood of rabbit or human blood contains two important factors viz factor-X and factor-V, which are necessary for the growth of type species of *H. influenzae* (2). Factor-X is a heat stable substance, the hemin associated with haemoglobin, whereas factor-V is a heat labile coenzyme Nicotinamide Adenine Dinucleotide (NAD). Other nutrients such as nitrogen compounds are supplied by peptic digest of animal tissue and beef extract incorporated in the medium. Sodium chloride helps to maintain osmotic balance of the medium. Pathogenic *Haemophilus* species may be presumptively identified by determining *in vitro* growth requirements for X and V factors and by haemolytic reactions.

## Quality Control

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 2.0% agar gel.

**Colour and Clarity of prepared medium**
Basal medium : Light yellow coloured clear to slightly opalescent gel After addition of blood & heating : Chocolate brown coloured, opaque gel forms in Petri plates

**Reaction**
Reaction of 4.5% w/v aqueous solution at 25°C. pH : 7.6±0.2

**pH**
7.40-7.80

**Cultural Response**
M472: Cultural characteristics observed with added sterile rabbit or human blood, under 5-10% CO2 and 70% humidity, after an incubation at 35-37°C for 18-24 hours .

| Organism | Inoculum (CFU) | Growth | Recovery |
|---|---|---|---|
| **Cultural Response** | | | |
| *Haemophilus influenzae* ATCC 35056 | 50-100 | luxuriant | >=70% |
| *Staphylococcus aureus* ATCC 25923 | 50-100 | luxuriant | >=70% |
| *Streptococcus pyogenes* ATCC 19615 | 50-100 | luxuriant | >=70% |

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

1. Sell S. H., Wright P. F., (Eds.), Haemophilus influenzae, Epidemiology, Immunology, and Prevention of Disease, Elsevier Biomedical, New York, 1982, St. Geme J. W.,III, Falkow S: Infect and Immun, p.4036, 1990
2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.

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