



Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Levinthals Medium Base

Product Code: DM 1472

Application: - 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 (25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The genus Haemophilus includes a number of species that are responsible for a wide variety of infections but share a common morphology and requirement for blood-derived factors during their cultivation in the lab. Haemophilus influenzae, the major pathogen, is the most virulent organism in this group, causing bloodstream invasion and meningitis in children below 2 years of age. 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, for growth: are -X and -V factor⁽¹⁾. Levinthals Medium is used for the cultivation of Haemophilus those require haemoglobin for their growth in the culture medium.

Whole blood of rabbit or human blood contains two important factors viz x and v factor necessary for the growth of different of H. influenza Species⁽²⁾. Factor-X is a heat stable substance in which the hemin is associated with haemoglobin, where as factor-V is a heat labile coenzyme named as 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.

Methodology

Suspend 45 grams of powder media in 1000 ml distilled water. Shake well & 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.

Quality Control

Physical Appearance

Cream to yellow homogeneous coarse 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 range 7.10-7.50

Cultural Response/ characteristics

DM1472: 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.





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Organism	Inoculum (CFU)	Growth	Recovery
<i>Haemophilus influenzae</i> ATCC 35056	50-100	luxuriant	>=70%
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant	>=70%
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	>=70%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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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