Levinthal's Medium Base

M472

Intended Use:

Recommended for cultivation of *Haemophilus* species.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM peptone B #	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

Equivalent to Beef extract

Directions

Suspend 45 grams in 1000 ml purified / 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 45-50°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 peptone and HM peptone B 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.

Type of specimen

Bkhmhb`k r` l okdr 9 Sotst l , cerebrospinal fluid

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1-2).

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

Warning and Precautions

In Vitro diagnostic 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.

imitations

1. Some *Haemophilus species will grow on Bordetella isolation media and cross-react with B. pertussis antisera.*
2. *B. pertussis colonies may not be visible without the aid of a microscope for 2-4 days.*

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
Cultural characteristics observed with added sterile rabbit or human blood, under 5-10% CO_2 and 70% humidity, after an incubation at 35-37°C for 18-24 hours .

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

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.

Revision : 1 / 2011