Product :
LöWENSTEIN JENSEN MEDIUM BASE

Specification
Medium for the detection and enumeration of *Mycobacterium ssp.*

Formula * in g/L
Monopotassium phosphate..... 2,40
Magnesium Sulphate..... 0,24
Magnesium Citrate 0,60
Asparagine (L)..... 3,60
Potato Starch.....30,00
Malachite green (Oxalate)..... 0,40

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 37,2 g of the powder in 600 mL of distilled water to which 12 mL of glycerol have been added. (Do not add glycerol if glycerophobic organisms are to be cultivated). Bring to the boil and sterilize by autoclaving at 121ºC for 15 minutes. Cool to 50ºC. Add 1 litre whole-egg homogenate prepared from fresh hen eggs under sterile conditions; Adjust the pH to 6.8 and stir to give a homogeneous mixture avoiding formation of bubbles. Distribute in suitable sterile containers such as screw-capped tubes. Arrange containers in slanted position, then inspissate and coagulate at 85ºC for 45 minutes in an inspissator saturated with water vapour or in free-flowing steam. The culture medium should be heated once more in this way after about 24 hours to guarantee its sterility.

Description
Löwenstein originally formulated a medium for cultivation of mycobacteria in which congo red and malachite green were incorporated for the partial inhibition of other bacteria. The present formula, developed by Jensen, differs in the citrate and phosphate content, does not contain congo red and has an increased malachite green concentration.
Lowenstein-Jensen Medium Base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Glycerol (if required) and egg mixture are added prior to the inspissation process. These substances provide fatty acids and protein required for the metabolisms of mycobacteria. The coagulation of egg albumin during the sterilization provides a solid medium for inoculation purposes.

Technique
The sample must be treated according its origin and concentred if it is necessary. All the manipulations with the sample must be performed with the suitable safety standards.
Inoculate the culture medium massively by spreading the sample in the surface. Use the glycerol-free culture medium when culturing glycerophobic mycobacteria. Incubate for four weeks at 35ºC in horizontal position. After the hiding of the inoculum (2-3 days) the tubes are firmly tightened and aerated weekly. Typical colonial morphology requires a good oxygenation and absence of liquid in the surface. Check the tubes for colony growth after 10-14 days and then in weekly intervals. The final result is obtained after 8 weeks of incubation.
Appearance of colonies of *Mycobacterium tuberculosis*
on Lowenstein-Jensen Medium with Glycerol or not.
Type humanus (R variant)
- with glycerol
Eugonic growth: Abundant, raised, crumbly, dry, usually yellowish (navel form) colonies
- Glycerol-free
The same pattern but with a poorly growth
Type bovinus (S variant)
- with glycerol
Sparse growth or no growth at all
- Glycerol-free
Dysgonic growth: flat, moist, glossy, confluent colonies (often nipple form) without pigment formation.
Type gallinaceous y Tipo poikilothermorum
- with glycerol and Glycerol-free
Rapid growth in the form of a moist, fairly abundant "lawn".
Optimal temperature 25ºC
Optimal temperature 41-42ºC.

Quality control

Incubation temperature: 35ºC ± 2,0 **Incubation time:** 48h-20 days

Inoculum: Streaking inoculation

Microorganism	Growth	Remarks
Mycobacterium smegmatis ATCC 14468	Good	-
Mycobacterium kansasii ATCC 12478	Good	-

Mycobacterium spp

References

· JENSEN, K.A. (1932) Reinzüchtung und Typenbestimmung von Tuberkelbazillenstammen. Zbl. Bakt. I. Orig. 125:222 -239
· LOWENSTEIN, E. (1931) Die Züchtung der Tuberkelbazillen aus dem strömenden Blute. Zbl. Bakt. I. Orig. 120:127-129
· BALOWS A., W.J. HAUSLER JR , K.L. HERRMANN, H.D. ISENBERG, H. JEAN · SHADOMY (1991) Manual of Clinical Microbiology 5th ed ASM Press, Washington DC.
· PFYFFER G.E., B.A. BROWN-ELLIOT & R.J. WALLACE Jr. (2003) Mycobacterium: General Characteristics, Isolation, and Staining Procedures in Manual of Clinical Microbiology 8th ed. by Murray, Baron, Jorgensen, Pfaller and Yolken. ASM Press, Washington DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC and <60 % RH).

Packaging