



Kirschner Medium Base, Modified

M161

Kirschner Medium Base with added glycerol and enrichments is used for cultivation of *Mycobacterium tuberculosis* .

Composition**

Ingredients	Gms / Litre
Disodium phosphate	3.000
Monopotassium phosphate	4.000
Magnesium sulphate	0.600
Sodium citrate	2.500
L-Asparagine	5.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.1 grams in 700 ml distilled water. Add 200 ml glycerol. Heat to boiling to dissolve the medium completely. Dispense in 9 ml aliquots. Sterilize by autoclaving at 115°C for 15 minutes. Just before use, aseptically add 1 ml of Horse serum (RM1239) and 100 IU Penicillin per 9 ml medium.

Principle And Interpretation

Mycobacterium tuberculosis is an acid-fast gram-positive aerobic bacteria involved in most cases of tuberculosis. Humans are the only reservoir for the bacterium. Many non-pathogenic Mycobacteria are components of the normal flora of humans, found most often in dry and oily locales. Kirschner Medium was first developed by Kirschner based on the formulation of Longs Medium (1) and further modified with addition of glycerol and enrichments for the cultivation of *M.tuberculosis* . It is widely used for antibacterial test, for antituberculosis agents and sometimes in differential culture of *M.tuberculosis* from unhealthy materials. Kirschner Agar Medium is made by addition of agar (2%) to this medium. Kirschner Semisolid Agar Medium is obtainable by addition of agar upto 0.1%. In case of screening test for antituberculosis agents on solid media, it takes at least 3-4 weeks to achieve culture of tubercle bacilli. Broth medium can give results in a week or two; hence broth medium is widely used in cases where rapid results are needed.

Kirschner medium contains two phosphates, a sulphate and citrate, which buffer the medium. Hence the medium can be directly inoculated without any prior neutralization. L-asparagine in the medium supports the growth of *M.tuberculosis* , as it is a good nutrient for the organism. Horse serum also promotes the growth of the organism. Penicillin inhibits the growth of contaminating bacteria. At first stage after inoculation of *M. tuberculosis* , granular colonies are observed at the bottom of the tube and as the incubation proceeds, bacterial film will be formed on the surface, rendering the medium transparent.

Quality Control

Appearance

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless solution having slight white precipitate at the bottom.

Reaction

Reaction of 1.51% w/v aqueous solution (with Glycerol) at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M161: Cultural characteristics observed with added Horse Serum (RM1239) and 100IU Penicillin, after an incubation at 35-37°C for 2-4 weeks.

Organism	Inoculum (CFU)	Growth
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<i>Mycobacterium tuberculosis</i> H37 RV(25618)	50-100	good-luxuriant
<i>Mycobacterium smegmatis</i> ATCC 14468	50-100	good-luxuriant
<i>Mycobacterium fortuitum</i> ATCC 6841	50-100	good-luxuriant

Storage and Shelf Life

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

Reference

I.Baker F.J. and Breach M.R., 1980, Medical Microbiological Techniques, Butterworths and Co. Ltd.

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