Kirschner Medium Base, Modified

M161

Intended Use:

Recommended for cultivation of *Mycobacterium tuberculosis* from clinical samples.

Composition**

Ingredients	g / L
Disodium hydrogen phosphate	3.000
Potassium dihydrogen 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 purified/distilled water. Add 200 ml glycerol. Heat to boiling to dissolve the medium completely. Dispense in 9 ml aliquots. Sterilize by autoclaving at Δ 115°C for 15 minutes. Just before use, aseptically add 1 ml of Horse serum (RM1239) and 100 IU Penicillin per 9 ml medium.

Δ corresponds to 10 lbs pressure.

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.

Type of specimen

Clinical samples : Sputum

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).

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

Warning and Precautions

In Vitro diagnostic use only. For professional 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.

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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

Organism	Growth
Mycobacterium tuberculosis H37 RV(25618)	good-luxuriant
Mycobacterium smegmatis ATCC 14468	good-luxuriant
Mycobacterium fortuitum ATCC 6841	good-luxuriant

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Baker F.J. and Breach M.R., 1980, Medical Microbiological Techniques, Butterworths and Co. Ltd.
2. Isenberg, (Ed.), Clinical Microbiology Procedures Handbook 2nd Edition
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

IVD *In vitro* diagnostic medical device

CE CE Marking

30°C 10°C Storage temperature

Do not use if package is damaged