# Mycocult®

## Intended Use
Mycocult® is a ready to use Lowenstein Jensen solid medium for *Mycobacterium tuberculosis* isolation.

## Summary
Infection with *Mycobacterium tuberculosis* remains a major public health problem. The epidemic of Tuberculosis and Multi Drug Resistant Tuberculosis reflects the failure of public health and social program's towards prompt treatment of infected cases and screening of high-risk population. Culture, isolation and sensitivity of *Mycobacterium tuberculosis* from patient groups using standard culture methods remain the gold standard for *Mycobacterium tuberculosis* detection and effective and swift treatment worldwide.

## Principle
Lowenstein Jensen medium supports the growth of *Mycobacterium tuberculosis*. The glycerol present in the medium enhances the growth of *Mycobacterium tuberculosis*. Accurate amount of malachite green not only has an inhibitory effect on growth of organisms other than *Mycobacterium tuberculosis*, but also provides the desired colour contrast for easy identification of *Mycobacterium tuberculosis* colonies.

## Reagent
Microxpress® Mycocult® is a reagent for laboratory use only.
Mycocult® Lowenstein Jensen medium is provided as a ready to use slant.
It is a standard non-selective inspissated egg based solid medium for the isolation of *Mycobacterium tuberculosis* from biological specimen such as sputum, CSF, urine.

## Additional Material Required
Sterile plating loops (10 µL), incubator at 35°C-37°C, biosafety hood with Bunsen burner, activated 2% glutaraldehyde solution, 0.2 mL micropipettes.

## Specimen Collection and Preparation
Collect specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antitubercular drugs.
**CSF:** Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen.
**Body fluids:** Disinfect the site and collect specimen with aseptic precautions.
**Sputum:** Collect 5 to 10 mL in a sterile container from an early morning specimen of deep productive cough. For induced specimen use sterile saline. Have patients rinse mouth with water to minimize specimen contamination with food particles, mouthwash or oral drugs.
**Urine:** As organisms accumulate in the bladder overnight, first morning void provides best yield. Collect midstream clean catch urine, first morning catheterization/ suprapubic taps in sterile containers.

## Specimen Preparation
Proper decontamination and concentration of specimen containing normal microbial flora are crucial to detection of *Mycobacterium tuberculosis*. Specimen obtained from sterile sites such as CSF, peritoneal or pleural fluids do not need decontamination. However, since most specimens for AFB smear and culture are from respiratory tract and mucous traps AFB and protects other organisms from decontamination and concentration, decontamination and liquefaction is a must. Most satisfactory for this purpose is a combination of N-Acety-L-Cysteine (mucolytic agent) and 2% NaOH (decontaminant)-(available as Lyfectol® from Microxpress®). Petroffs method of decontamination can also be used.

### Preparation of Water Tween Solution
1. To 10 mL of sterile distilled water add 40 µL of sterile tween 80 solution.
2. Mix thoroughly by shaking in a swirling direction or by vortexing to homogenise the solution.
3. Use this solution for preparation of dilution.

## Directions
1. Bring the Lowenstein Jensen medium slant to room temperature.
2. Label the L.J. medium slant appropriately.

3. Draw 10 µL of the decontaminated and concentrated specimen from the reconstituted pellet with a sterile calibrated loop and plate it on the L.J. medium slant aseptically.
4. For quantitative evaluation prepare bacterial suspension with sterile water tween solution to match McFarland 0.5 standard, dilute this further with sterile water tween solution 1:10000 and Seed 100 µL on the Lowenstein Jensen medium slant aseptically (seed stock consists of approx.-15000 organisms/mL).
5. Close the L.J. slant cap tightly and incubate at 35°C-37°C.
6. Observe for growth weekly till 8 weeks.

## Quality Control

**Appearance:** Bluish green coloured, opaque, smooth slant.
**Cultural Response:** Cultural characteristics observed after an incubation of 2-4 weeks at 35°C-37°C.

| Organism | Growth | Colony Characteristics |
|---|---|---|
| *Mycobacterium tuberculosis* H37Rv Strain | Good | Granular, rough, warty, dry, friable colonies |

## Interpretation of Results

*Mycobacterium tuberculosis* colonies may be detected from third week onwards up to eight weeks. The colonies are characterized by rough granular buff coloured growth, which has an initial size of 1-3 mm and full-grown size of 58 mm.

## Remarks

1. Discoloured, dislodged, or contaminated medium should not be used.
2. Improper decontamination and concentration procedure will yield erroneous results.
3. Treat the specimens and used slants by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.
4. Good laboratory practices and hazard precautions must be observed at all times.
5. In specimens from patients already on antitubercular drugs, the initial growth may be further delayed.
6. Growth on the Lowenstein Jensen slant within the first week post inoculation usually indicates atypical *Mycobacterium* or contamination due to insufficient decontamination of specimen.
7. All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

## Storage and Stability

1. Store the Mycocult® kit at 2°C-8°C, away from light.
2. Stability of the unopened medium is as per the expiry date mentioned on the label.
3. Avoid jerks and vibration while storage, shipping and incubation.
4. Upon opening, the medium must be put into use instantly.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference


1. Clinical Diagnosis & Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17th Edition 1998, Edited by John Bernard Henry.
2. Tuberculosis; A Clinical Handbook, 1st Edition 1995, Edited by L.I. Lutwick.
3. Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Duguid.
4. Microbiology, Zinsser, 16th Edition 1976, Edited by W.J. Joklik, H.P. Willet.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.


## Product Presentation:

| Cat. No. | Product Description | Pack Size |
|---|---|---|
| 203130860006 | Ready Prepared Slants | 6 Slants |