Mycocult® PY

Intended Use
Mycocult® PY is a ready to use Lowenstein Jensen solid medium with sodium pyruvate for *Mycobacterium bovis* isolation.

Summary
Infection with *Mycobacterium bovis* remains a major public health problem. The epidemic of bovis and Multi Drug Resistant bovis 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 bovis* from patient groups using standard culture methods remain the gold standard for *Mycobacterium bovis* detection and effective and swift treatment worldwide.

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

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

Additional Material Required
Sterile plating loops (10 µL), incubator at 37°C±0.5°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 bovis*. 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.

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 37°C±0.5°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 on L.J. Control Slant	Colony Characteristics on L.J. Control Slant	Growth on Mycocult® PY Slant
Mycobacterium tuberculosis H37Rv Strain	Good	Granular, rough, warty, dry friable colonies	Partial Inhibition

Note:

1. *Mycobacterium bovis* is closely related taxonomically to *M. tuberculosis* and belongs to the *M. tuberculosis* complex.
2. On L.J. slant, *M. bovis* produce small, granular, rounded, non-pigmented colonies with irregular margins.
3. Most strains of *M. bovis* are niacin negative, do not reduce nitrate and do not grow in the presence of thiophene-2 carboxylic acid hydrazide (T2H), characteristics that distinguish the species from most strains of *M. tuberculosis*.

Interpretation of Results

Mycobacterium bovis colonies may be detected from third week onwards up to eight weeks. The colonies are characterized by tiny transparent growth which later becomes white.

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® PY 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
203130870006	Ready Prepared Slants	6 Slants

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.