Combicult®

Intended Use
Combicult® is a ready to use, combipack of solid and liquid 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
The gold standard for primary isolation of *Mycobacterium tuberculosis* is the use of liquid media in conjunction with solid media. Most *Mycobacterium* species grow more quickly in liquid media than solid media. Liquid media also support higher detection rates especially with specimen material containing smaller number of bacilli. Simultaneous inoculation of solid media and liquid media yields significantly higher recovery rates for *Mycobacterium tuberculosis* growth as compared to when each media is used independently.

Reagent
Microxpress® Combicult® is a reagent for laboratory use only.
Lowenstein Jensen medium is provided as a ready to use slant. Kirchner medium is provided as a three-component medium:

a) Kirchner medium base.
b) Kirchner selective enrichment containing antibiotic cocktail Polymyxin B, Amphotericin B, Carbenicillin, Trimethoprim (PACT)
c) Sterile distilled water for reconstitution of Kirchner selective enrichment.

Lowenstein Jensen medium is a standard non-selective inspissated egg based solid medium for the isolation of *Mycobacterium tuberculosis* from biological specimen such as sputum, CSF, urine. Lowenstein Jensen medium supports the growth of *Mycobacterium tuberculosis*. The glycerol present in the Lowenstein Jensen 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* but also provide the desired colour contrast for easy identification of *Mycobacterium* colonies. Kirchner medium is a liquid medium enriched with serum. Kirchner medium has Polymyxin B, Amphotericin B, Carbenicillin, Trimethoprim as inhibitory antibiotic cocktail for most of the bacteria and fungus other than *Mycobacterium*. Being a buffered medium, it allows direct inoculation of larger inoculum up to 500 µL, and also keeps up the acid base balance during the growth phase.

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.

Directions
Kirchner Medium

1. Bring the Kirchner to room temperature.
2. Label the Kirchner medium appropriately.
3. Draw 10 µL of the decontaminated and concentrated specimen from the reconstituted pellet with a sterile calibrated loop.
4. Inoculate in Kirchner medium aseptically.
5. Close the Kirchner medium cap tightly and incubate at 37°C±0.5°C.
6. Observe for growth every third day till 8 weeks.

Lowenstein Jensen Slant:

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 specimens from the reconstituted pellet with a sterile calibrated loop and plate it on the L.J. medium slant aseptically.

4. For quantitative evaluation prepare bacteria suspension to match McFarland 0.5 Standard, dilute this 1:10000 and seed 100 µL on the L.J. medium slant aseptically (seed stock consist of approximately 15000 organisms/mL).
5. Close the L.J. slant cap tightly and incubate at 37°C±0.5°C.
6. Observed for growth weekly till 8 weeks.

Quality Control

Appearance:

L.J. Slant- Bluish green coloured, smooth slant.
Kirchner Selective Enrichment- Reddish orange coloured, lyophilised material.
Kirchner Medium Base- Clear, Red coloured liquid.
Sterile Distilled Water- Clear, colourless liquid.

Cultural Response: Cultural characteristics observed after an Incubation of 2-4 weeks at 35°C-37°C.

Organisms (ATCC)	**Results**
Mycobacterium tuberculosis H37Rv Strain	Good
Staphylococcus aureus subsp. *aureus* (25923)	Inhibited

Interpretation of Results

1. *Mycobacterium tuberculosis* colonies on L.J. slant may be detected from third week onwards up to eight weeks. The colonies are characterised by rough granular buff coloured growth, which has initial size of 1-3 mm and full-growth size of 5-8 mm.
2. *Mycobacterium tuberculosis* growth in Kirchner medium is characterised by fluffy growth to small granules. The granules sediment to the bottom.
3. Since both the media differ in their composition, growth of *Mycobacterium tuberculosis* in either medium should be considered as a positive culture results, the growth needs to be identified.

Remarks

1. Discoloured, dislodged, contaminated or turbid medium should not be used.
2. Improper decontamination and concentrated procedure will yield erroneous results.
3. Good laboratory practices and hazard precaution must be observed at all times.
4. While observing growth in liquid medium, care needs to be taken to differentiate between *Mycobacterium* growth and specimen material's own turbidity.
5. Treat the specimens and used slants by immersing in 2% activated glutaraldehyde for at least two hours before incineration and disposable.
6. Preparation of Kirchner medium has to carried out prior to inoculation of specimens or culture.
7. In specimens from patient, already on antitubercular drugs the initial growth may be further delayed.
8. Growth on the L.J. slant / Kirchner medium within the first week post inoculation usually indicates atypical *Mycobacterium* or contamination due to insufficient decontamination of specimen.
9. All culture growth should be characterized on morphology, AFB stain and biochemical tests.

Storage and Stability

1. Store the Combicult® kit at 2°C-8°C, away from light.
2. Stability of the unopened media is as per the expiry date mentioned on the label.
3. Avoid jerks and vibrations while storage, shipping and incubation.
4. Upon opening, the media 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. Biotest Bulletin 6:45-50 (1998).
4. AM Rev. of Respiratory Diseases, 1990, 142: 725-735.

5. Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Duguid.
6. Microbiology, Zinsser, 16th Edition 1976, Edited by W.J. Joklik, H.P. Willet.
7. Cultural Detection of Mycobacteria, L. Naumann, Biotest Bulletin 5:177-180 (1995).
8. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203030360001	Combicult®	One set

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.