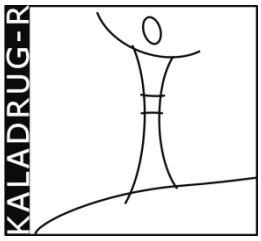
KALADRUG-R: Clinical SOP#D

Isolation of parasites from PKDL tissue lesions



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A. Materials required

1. Medium M199 (Sigma)
2. RPMI 1640 Amino acids solution (50 X) (Sigma, Cat no. R7131)
3. RPMI 1640 Vitamin solution (100 X) (Sigma, Cat No. R7256)
4. Heat inactivated fetal bovine serum
5. 15 ml sterile tubes/ sterile flask 25 cm^2
6. Sterile micropipettes
7. Sterile micropipettes tips
8. Tissue Biopsy Punch
9. 24 no. Surgical sterile blade
10. Scalpel
11. Forceps (Fine and broad end)
12. Petri dishes (90 mm)

B. Preparation of Nutrient medium (500 ml)

1. Prepare Medium M199
2. Add 10ml RPMI 1640 Amino acids solution in medium M199, to make final concentration 1X.
3. Add 5 ml RPMI 1640 Vitamins solution in medium M199, to make final concentration 1X.
4. Make total volume 500ml.
5. Store the prepared nutrient medium at 4°C.

C. Procedure for isolation of parasites from PKDL tissue

1. Take fresh tissue punch biopsy from nodular or macular region in Nutrient medium and carry to the Laminar Air Flow. Process the sample immediately.
2. Give tissue sample a brief wash with ethanol in a Petri dish for surface sterilization.
3. Transfer the tissue in petridish containing M199, and give tissue two washes in M199 with help of forceps.

4. Chop the tissue into small pieces with help of sterile blade and add pieces to a sterile 15 ml tube/flask containing 5 ml Nutrient medium with 30% FCS.
5. Transfer the tube to BOD incubator at 26°C.
6. Observe periodically under microscope starting after 1 week, till promastigotes appear.
7. Once sufficient number of promastigotes appear, subculture in M199 containing 10% FCS.

Note: All steps to be done in Laminar air flow.