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| A Comparative Multicentric Non-Infireority Clinical Trial of WHOMBMDT with a New Monthly Chemotherapy Regime containing Rifampicin, Moxifloxacin and Clarithromycin (RMC) on Multibacillary patients from IndiaStandard Operating Procedure 6Sample collection and transport ) | | | |
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**1.** **PURPOSE**

This document describes the process of collection, preparation and examination of Molecular viability assay (MVA) samples taken from patients for the RMC study.

**2. Background**

Current WHOMDT does not kill 100% bacteria even after a full course of treatment in a subset of patients harboring a large bacterial load thus continuing transmission of the disease responsible for endemicity in some countries. The duration of MDT is long and promotes noncompliance. MDT continues to be controversial with limited evidence support resulting in multiple reformulations since the last 40 years. This calls for a search for newer, more efficacious drugs with shorter duration of action evidenced with well-designed clinical trials. Relapse, advocated as the key outcome measure of efficacy of MDT, has its drawbacks. Relapse studies require long years of follow up. The gold standard test for viability was Mouse foot pad studies which is costly and time consuming. Hence, we propose Molecular Viability Assays as outcome measure of efficacy which are newer and better techniques to test viability faster.

In this study, we propose to conduct a Randomized Controlled study comparing WHO MBMDT with a monthly regime consisting of currently most bactericidal and safe drugs of Rifampicin, Moxifloxacin and Clarithromycin in MB leprosy patients.

**3. SCOPE**

This document applies to all staff involved in collection, preparation and examination of Molecular viability assay (MVA) samples.

**4. PROCEDURE**

Skin biopsy plays a crucial role in the clinical process, being routinely essential for histopathological diagnosis in leprosy. It holds significant importance in accurately classifying histopathological features, determining bacillary index, monitoring treatment response, and assessing disease activity. Additionally, it aids in distinguishing between relapse and reversal reaction and categorizing reactions into type 1 or type 2, thus enhancing diagnostic precision and treatment management.

*4.1* *Site selection*

* The area from where the biopsy is to be taken should be active and representative of the manifestations of leprosy with the consultation of clinician. Facial patches should be avoided. Bodily patches are preferred.
* For example:

1. Hypo pigmentated patch- biopsy from the centre of the lesion.
2. Annular plaque - biopsy from active spreading edge.
3. Patch with central clearing - - biopsy from active spreading edge.
4. Type 1 reaction patch – edge preferred ( to compare with normal skin)
5. Nodule – biopsy the nodule
6. Type 2 reaction – Take from non reactional skin ( infiltration )

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*4.2* *Site Preparation*

* Universal precautions should be observed in obtaining skin biopsy.
* Any common skin antiseptic such as povidone-iodine solution or can be used to prepare the biopsy site.
* The area from where the biopsy is to be taken should be marked with a skin marker by the physician
* 2% lignocaine 1ml intradermally can be used as anaesthetic agent for the biopsy

*4.3* *Skin Biopsy*

* The 4 mm punch should be removed from sterile packaging and the sharp metal tip must be kept sterile by avoiding contact with non-sterile surfaces.
* The punch should be kept perpendicular to the skin and gently pressed down onto the skin
* A gentle twisting motion in one direction with slight downward pressure should be applied to cut through all the layers of skin including epidermis, dermis, and the most superficial parts of the subcutaneous fat.
* The tool should be gently pulled out at a 45-degree angle, avoiding damage to the sample.
* Tweezers or a 25-gauge needle should be used to gently grasp the biopsy and pull the sample up and out and the skin piece should be immediately transferred to biopsy vial of 10% buffered formalin. Second biopsy should be taken from the same site and transfer the biopsy in RNA later vial.
* Separate biopsies should be taken for HP and MVA
* Histopathology sample tissue is fully submerged in 10% formalin an MVA sample in RNAlater. The cap should be closed tightly and seal the lid with parafilm
* A hemostatic agent should be applied to stop the bleeding and antibiotic ointment to sampling area. For larger biopsies, a suture may be needed.

Alcohol-resistant marker should be used to label the biopsy vial with an identifier that matches ***exactly*** what is indicated on the specimen form. The screening ID should be labelled on the vials.

* About 3 inches of newspaper or bubble wrap can be used to ensure that the vials are safely shipped to TLM Shahdara.

*4.4* *Molecular Viability Assay*

* **RNA Extraction:** Extraction of RNA from tissue using trizol method or RNA extraction Kit (Qiagen).
* RNA samples will be quantified by Qubit Fluorometer.
* cDNA will be prepared.
* qPCR will be done for the amplification of 16S rRNA, esxA and hsp18 gene to check the viable load of *M. leprae*.
* Absolute quantification will be done to calculate the copy number of each gene.

The following table must be completed and sent along with the transported samples each time of transport.

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| **Screening ID** | **Date sent** | **Sample sent for HP** | **Sample sent for MVA** |
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