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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 5Skin Biopsy and Histopathology | | | |
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**1.** **PURPOSE**

This document describes the process of collection, preparation and examination of Slit skin smears 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 skin biopsy 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. For example:

Small hypo pigmentated patch- biopsy from the centre of the lesion.

Annular Macule- biopsy from active spreading edge.

Multiple lesions with different morphology- more than one biopsy should be taken.

*4.2 Site Preparation*

* Universal precautions should be observed in obtaining skin biopsy.
* Any common skin antiseptic such as isopropyl alcohol, povidone-iodine solution or chlorohexidine gluconate 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 6mm biopsy tool should be removed from sterile packaging and the sharp metal tip must be kept sterile by avoiding contact with non-sterile surfaces.
* The biopsy tool 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 RNAlater vial.
* It should be ensured that the tissue is fully submerged in 10% formalin and/or RNAlater. The cap should be closed tightly and seal the lid with parafilm. ***Each biopsy should be placed in a separate vial*.**
* A hemostatic agent should be applied to stop the bleeding and antibiotic ointment to sampling area. For larger biopsies, a suture may be needed.
* An an alcohol-resistant marker should be used to label the biopsy vial with an identifier that matches ***exactly*** what is indicated on the specimen form. Check to make sure that each vial is easy to identify with the information provided on the form.

***Required information to include on the form****:*

* *Patient ID*
* *Name of study site*
* *Date of tissue collection*
* *Type of lesion*
* *Any other important information*
* A thin layer of Parafilm should be added around the vial covering the seal.
* About 3 inches of newspaper or bubble wrap can be used to ensure that the vials are safely shipped to TLM Shahdara.

*4.4* *Histopathology*

* All the collected biopsies should be clearly labelled with patient information/code.
* Pack the collected biopsy properly and send to the lab for histopathological investigation.
* Tissue processing is the crucial step that prepares the tissue for microscopic analysis. It involves several stages:
* **Fixation:** The tissue is chemically preserved using fixatives (e.g., formalin) to prevent decay and maintain cellular structures.
* **Dehydration:** Water is removed from the tissue using a series of alcohol solutions.
* **Clearing:** The dehydrated tissue is cleared using a solvent (e.g., xylene) to make it transparent.
* **Infiltration:** The tissue is impregnated with paraffin wax, which provides support for sectioning.
* **Embedding:** The tissue is embedded in paraffin blocks, allowing thin sections to be cut.
* **Section Cutting:** Microtomes are used to cut thin (usually 5 microns thick) sections from the paraffin-embedded tissue.
* **Staining and Labeling:** The sections are stained (e.g., with hematoxylin and eosin) to highlight cellular structures.
* **Mounting:** Stained sections are permanently mounted on glass slides.
* Once the slides are prepared, they are sent to the pathologist for examination and diagnosis.