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| A Comparative Multicentric Non-Inferiority Clinical Trial of WHOMBMDT with a New Monthly Chemotherapy Regime containing Rifampicin, Moxifloxacin and Clarithromycin (RMC) on Multibacillary patients from IndiaStandard Operating Procedure 4Collection, Preparation and Examination of Slit Skin Smear | | | |
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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 Slit skin smear samples.

**4. Procedure**

The smear is a means of estimating the number of acid-fast bacteria present, reported as the Bacterial Index (BI). It is important in determining the type and severity of disease as well as assessing the response to treatment.

*4.1 General considerations*

* Initial skin smears should usually be taken from 6 “routine sites” (both earlobes, elbows, and forehead) as well as several typical lesions from the patient.
* Repeat smears should be obtained from 3 to 4 of the most active sites previously tested to evaluate progress.
* All microscopic slides on which skin smears are made should be pre-cleaned in 70% alcohol. The slides are wiped dry with a clean lint free tissue paper/cloth.
* Slides should be air-dried and NEVER heat fixed.
  1. *Procedure for Obtaining Smears*
* Universal precautions should be observed in obtaining skin smears.
* The skin should be cleansed with 70% alcohol and air-dried or wiped dry with cotton.
* A fold of skin should be made relatively avascular by pinching or mild clamping. If the skin cannot be grasped by pinching, it can be compressed. A surgeon's glove may aid in grasping. Local anaesthesia is generally unnecessary. The compression of the skin by pinching aids in the anaesthesia.
* An incision 3-5 mm long and 2-3 mm deep should be made A scalpel with a #15 Bard-Parker blade may also be used. Mild pressure to maintain relative avascularity should be continuously applied to the area until an adequate smear has been obtained.
* There should be no bleeding , If there in then wipe it off with a sterile cloth .
* After the incision is made, and before the blade is withdrawn, the inner surface of the wound should be scraped with the blade held at a right angle(90 degrees) to the incision. Upon scraping, with the 4 stroke method tissue fluid and dermal tissue are obtained.
* The material should be transferred to the cleaned microscope slide. A moderately thick smear, with a visible uniform opacity is made. The smear is made in a circular manner on the slide, no larger than 5-7 mm, beginning peripherally and ending in the centre, leaving a central “button” (2-4 mm) which can be easily focused upon with the microscope. Slides should be properly labelled as shown below in the sample diagram. Similar slides can be prepared for other sites.



* Tincture benzoin is used to seal the site of incision.
* A single technician should take all smears to ensure more uniform and consistent results.
  1. *Staining of Skin Smear*
* The slide with smear should be dried at room temperature. SHOULD NEVER BE HEAT FIX.
* The slides should be placed on a staining rack and flooded with 10% formalin fumes for 15 minutes for fixation.
* The slides should be gently rinsed well with tap water. The slides should be flooded with Ziehl-Neelsen carbol-fuchsin for twenty minutes. The carbol-fuchsin must be filtered before each use. Filtering can be accomplished by placing pre-cut filter paper strips on the slide prior to the addition of stain and left in place for the full twenty minutes.
* After removing and discarding filter paper strips, the slides should be gently rinsed well with tap water to remove excess stain.
* Decolorization with 2% acid alcohol for 1 minute should be done.
* The slides should be rinsed thoroughly with tap water.
* The slides should than be counterstained with alkaline methylene blue for 30 seconds to 1 minute.
* Finally the slides should be gently rinsed well with tap water and air dried.

**NOTE: Positive and negative control slides must be used each day for quality control purposes.**

*4.4 Microscopic Examination of Skin Smears*

* The stained smears should be examined with a quality microscope using the oil immersion objective (x100) to determine the total number of bacilli.
* The same individual should read all smears for the purpose of consistency. However, four separate quadrants of the smear are examined and averaged to establish the Bacterial Index.

*4.5 Reporting the Bacterial Index*

The results are reported on a 0 to 6+ semi-logarithmic scale using a descriptive phrase or numerical code. This is an indicator of the total bacillary load of the patient. It falls about 1 point per year during effective treatment as dead bacilli undergo lysis and are absorbed. In case of negative smear - At least 200 fields should be covered in four quadrants.

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| Very Numerous | ( +6 ) | over 1000 bacilli per oil immersion field. |
| Numerous | ( +5 ) | 100 to 1000 bacilli per oil immersion field. |
| Moderate | ( +4 ) | 10 to 100 bacilli per oil immersion field. |
| Few | ( +3) | 1 to 10 bacilli per oil immersion field. |
| Very few | ( +2 ) | 1 to 10 bacilli per 10 fields. |
| Rare | ( +1) | 1 to 10 bacilli per 100 fields. |
| None found | ( NF ) | No AFB seen on entire site. |

**Appendix 1**

Z-N Carbol Fuchsin Stain:

Basic fuchsin --------------------------- 1.0 gm.

Phenol crystals (melted)----------------5.0 mL.

95% ethanol --------------------------- 10.0 mL.

Water, to make ---------------------- 100.0 mL.

Dissolve stain in alcohol, and then add phenol/water mixture. Let stand overnight before use. Store in dark brown bottle. Stable for 1 year.

Acid alcohol:

Conc. HCl ------------------------------ 2.0 mL.

95% ethanol -------------------------- 98.0 mL.

Alkaline Methylene Blue:

KOH (10%) -------------------------- 0.10 mL

Methylene blue ---------------------- 0.35 gm.

95% ethanol ---------------------------30.0 mL.

Water to make -----------------------100.0 mL.

Dissolve the stain in the alcohol, then add the KOH and water mixture and allow to sit overnight. Filter before use.