



Net Educational Video for Residents

# Modified Ziehl–Neelsen staining for *Mycobacterium leprae*

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## ABSTRACT

Modified Ziehl–Neelsen staining for *Mycobacterium leprae* is a time-tested technique in the evaluation of leprosy. The staining helps to classify clinical types of leprosy into those with low and high bacillary load. It is also useful in assessing the response to therapy, identifying defaulters, and differentiating relapse from late reversal reactions. In this net educational video, we demonstrate the technique of taking an ear lobe smear and performing the modified Ziehl–Neelsen staining for acid fast bacilli.

**Keywords:** Slit skin smear, Bacteriological index, Morphological index, *Mycobacterium leprae*, Acid fast bacilli

## INTRODUCTION

Demonstration of *Mycobacterium leprae* (*M. leprae*) in slit skin smear (SSS) is a time-tested technique in the diagnosis, classification and management of leprosy. Demonstration of acid fast bacilli (AFB) in a skin smear is one of the three cardinal signs of leprosy.<sup>[1]</sup>

In 1963, Wade introduced the current technique of SSS.<sup>[2]</sup> Ziehl–Neelsen staining was introduced by Paul Ehrlich.<sup>[3]</sup> The staining method was modified by two doctors, the bacteriologist Franz Ziehl and the pathologist Friedrich Neelsen and was named after them.<sup>[4]</sup> Ziehl–Neelsen staining requires a primary stain (concentrated carbol fuchsin, basic fuchsin dissolved in phenol), a decolorizer, and a counter stain (Loeffler's methylene blue). This technique is also called hot staining method as heat is used to drive primary stain into waxy cell wall of this difficult to stain organism.<sup>[4]</sup> Modified Ziehl–Neelsen staining is ideal for demonstration of *M. leprae* as the standard Ziehl–Neelsen method can easily decolorize its mycolic acid coating.<sup>[5]</sup>

## INDICATIONS

- All suspected cases of leprosy
- To assess the treatment response in leprosy
- To diagnose relapse in treated, smear positive leprosy cases

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## PROCEDURE

### Materials needed

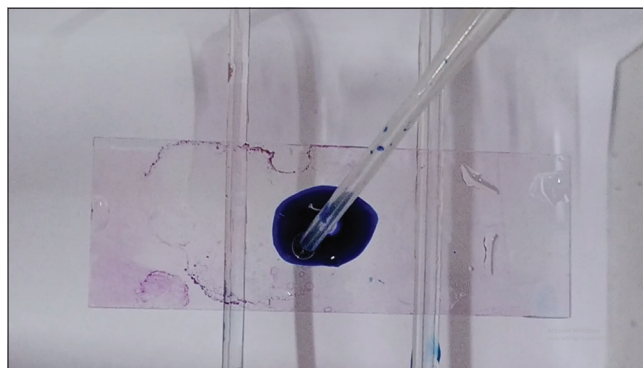
Spirit and cotton balls, gloves, glass slides, scalpel blade (Bard-Parker blade no:15), Bunsen burner or spirit lamp, glass rods, wash bottle, microscope, concentrated carbol fuchsin, Loeffler's methylene blue, 5% sulfuric acid, or acid alcohol mixture (1% hydrochloric acid and 70% ethanol).

### Collection of skin smears

- Site is cleaned with spirit.
- Skin is gripped between the thumb and the forefinger of the left hand to drive out blood.
- A 5 mm long and a 3 mm deep incision is made with size 15, Bard-Parker blade (with pressure of finger maintained). Incision should be deep enough to include the dermis.
- Turn the blade at right angles and scrape the wound several times in the same direction so that tissue fluid and pulp collect on one side of the blade.<sup>[5]</sup>
- Smear the collected material on a glass slide in a circular motion from inside to outside to an approximate size of 8 mm.<sup>[6]</sup>
- Two or more smears can be made on a glass slide.<sup>[5]</sup>
- Fix the smear over a flame.
- Record the body sites from which the smears are collected so that on follow-up, smears can be collected from the same sites.
- Do not expose the slide with the smear to sunlight, dust, or extremes of temperature as it may interfere with the uptake of stain.
- Long storage of fixed slides can also interfere with staining.

### Modified Ziehl–Neelsen staining for *M. leprae*

- Cover the slide with concentrated carbol fuchsin and apply heat beneath it, either with a Bunsen burner or a spirit lamp.
- Heating should be sufficient to rise steam from all sides; avoid boiling.
- Keep carbol fuchsin for 15 minutes.
- Stain is tipped away and the slide is kept under gentle stream of water.
- Pour acid-alcohol mixture or 5% sulfuric acid into the slide. Decolorize until the slide is faintly pink and then wash in running water.
- Cover the slide with the counterstain (1% methylene blue) for 10 seconds.
- Wash in running water and allow to dry [Video 1].
- Examine the smear under the oil immersion lens of the microscope.<sup>[5]</sup>



**Video 1:** Modified Ziehl–Neelsen staining for *Mycobacterium leprae*.

**Video available online at:** [https://doi.org/10.25259/JSSTD\\_34\\_2022](https://doi.org/10.25259/JSSTD_34_2022)

## INTERPRETATION OF THE RESULTS

*M. leprae* are seen as red rods against a blue background. Viable bacilli are seen as uniformly stained rods or solid bacilli having a length 4 times greater than the breadth. The dead bacilli stain irregularly and appear granular or fragmented. The bacilli may be seen singly, in small groups or closely packed bunches called globi.<sup>[7]</sup>

SSS is used to determine bacteriological index, (BI) morphological index (MI) and solid, fragmented and granular (SFG) index.<sup>[5]</sup>

SSS has a low sensitivity (10–50%); but a high (100%) specificity to diagnose leprosy.<sup>[8]</sup> Sensitivity is low at the tuberculoid end as the bacterial load is less. A negative smear does not exclude leprosy as at least 10,000 bacilli/g of tissue are needed to get a positive result.<sup>[9]</sup>

## CONCLUSION

Modified Ziehl–Neelsen staining of skin smears is a test of diagnostic and therapeutic significance in leprosy, which does not require any sophisticated equipment. A proper knowledge of the same is essential to obtain accurate results.

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### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

### Conflicts of interest

Dr. Mary Vineetha and Dr. Kidangazhiathmana Ajithkumar are on the editorial board of the Journal.

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