

## REPRINTED ARTICLES

*Articles published elsewhere which are considered by the Editorial Board to be of special interest are, with permission reprinted in full, or in condensed form, or in free translation.*

### PROCEDURE FOR DEMONSTRATING LEPROSY BACILLI IN PARAFFIN SECTIONS<sup>1, 2</sup>

G. L. FITE, M.D.

P. J. CAMBRE and M. H. TURNER, B.S.  
*Carville, La.*

The lesser degree of acid-fastness of leprosy bacilli as compared with tubercle bacilli appears in a disagreeable manner in the difficulty of demonstrating the organisms of leprosy in paraffinized tissues (1). Faraco (2) showed that by ordinary methods of demonstrating acid-fast organisms the leprosy bacilli are often not acid-fast, are not differentiated and may be stained by the counterstain. He devised a method of oiling the sections and staining with carbol-fuchsin while the sections contained oil. Under these conditions the bacilli retained this dye. The method is effective but awkward and cumbersome. In working with similar procedures it has been found that staining before removal of the paraffin is satisfactory (though impracticable) and that replacement of the paraffin with a light oil produces still better results. The following procedure, which is not exactly a new method, has proved most valuable in demonstrating leprosy bacilli in tissues, almost irrespective of the technics of fixation and embedding. It succeeds admirably with tissues indifferently fixed or embedded years previously where other procedures fail miserably. The use of picric acid in the fixative is contraindicated for this and other staining methods.

#### PROCEDURE

1. Remove paraffin with two changes of the following mixture, allowing a minute or two for each change:

Cottonseed (or peanut or olive) oil .....	1 part
Xylene .....	2 parts

<sup>1</sup> From the Pathology Laboratory, United States Marine Hospital (The National Leprosarium), United States Public Health Service.

<sup>2</sup> Reprinted, by permission, from the *Archives of Pathology*, 43 (1947) 624—5, with certain minor changes by the authors.—EDITOR

2. Drain, wipe off excess oil and blot to opacity. The residual oil in the section helps to prevent shrinkage and injury of the section.

3. Remove mercury crystals (if present) with strong solution of iodine, U.S.P. (two minutes), followed by a hyposulfite or thiosulphate solution rather than alcohol. Wash in tap water.

4. Stain cold five to ten minutes in any standard preparation of carbolfuchsin (Ziehl-Neelsen) but not in a concentrated solution such as Kinyoun's. Wash.

5. Decolorize with 1 per cent concentrated hydrochloric acid added to 70 per cent alcohol—not to the point of totality, but leaving a faint pink color. One to two minutes will be required. Wash.

6. Counterstain with Loeffler's alkaline methylene blue about thirty seconds. Wash in tap water.

7. Blot, let stand a few minutes to dry out well, mount directly in a synthetic mounting medium, such as "clarite" or "permount."

Almost any oil will serve the purpose, from liquid petrolatum to camphorated oil, although the volatile oils are less useful. The oil slows up the various steps a little, but not importantly. It hastens the acid-fast staining, however, so that the use of heat is preferably avoided, more even regular staining resulting without it. A larger proportion of oil in the xylene makes even decolorization difficult. It is possible to take sections to water by routine methods and then oil them, as Faraco did, but it is difficult to obtain even staining thereafter. The procedure as given is equally effective with the bacilli of rat leprosy and should do well with tubercle bacilli. It is particularly recommended for tissues containing the organisms of human leprosy.

#### REFERENCES

1. FITE, G. L.: *Am. J. Path.* **14**:491, 1938.
2. FARACO, J.: *Rev. brasil. leprol* **6**:177, 1938.