

✓ TO THE EDITOR:

The method for the counting of leprosy bacilli in lepromin routinely in use at the National Institute for Leprosy Research of Japan is to calculate the total number of the bacilli contained in each cubic centimeter of the bacterial suspension, on the basis of the bacterial count made on a standard smear. This smear is prepared by the use of a platinum loop of a certain specific size, spreading a loopful of the suspension over a circular area 1.5 cm. in diameter on the surface of a clean glass slide.

Before making such a count, a preliminary test is made employing some cultivable bacillus such as *E. coli*. For this purpose there is first made a uniform suspension of *E. coli* in a concentration of 1 mgm./cc., from which are made serial dilutions of 10^{-1} , 10^{-2} , 10^{-3} . Then a loopful of each dilution is smeared in a circle 1.5 cm. in diameter and, after staining, the number of the bacilli is counted. Several oil immersion fields are counted per smear, for the calculation of the mean count per field. The use of an eyepiece having radial sections has been found to be helpful for this counting. Separately, the same bacterial suspensions are cultured on agar plates for counts of viable bacilli per cubic centimeter.

When a suspension of leprosy bacilli (lepromin) is examined under the same conditions, the mean bacterial count per microscope field will give the total number of the bacilli contained in each cubic centimeter of the suspension by the following simple proportional equation:

Total number of bacilli per cc. = mean count per microscope field \times k, in which "k" is a constant dependent on the size of the loop.

$k = \text{count of viable } E. coli \text{ per cc.} / \text{mean count of } E. coli \text{ per field.}$

This counting method is by no means entirely precise for the counting of the number of leprosy bacilli in a suspension, but still it is a simple and practical measure quite sufficient for routine work. The following are the bacterial counts obtained on several lots of lepromin by the method described.

NILR lepromin, 1957	2271 $\times 10^4/0.1$ cc.
NILR lepromin, 1958	2234 $\times 10^4/0.1$ cc.
Lepromin of Wade, 1956	1505 $\times 10^4/0.1$ cc.

Details will be reported in the *La Lepro* by Kikuo Hayashi, Department of Bacteriology, National Institute for Leprosy Research.

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