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Immunodiagnostic Tests for Leprosy; a Need for Standards

To the Editor:

The immunodiagnosis of leprosy is becoming a realistic possibility with the advent of tests using epitopes specific to *Mycobacterium leprae* ([1–3]). A number of laboratories around the world are evaluating the role of IgM antibody against phenolic glycolipid-I (PGL-I) using the ELISA technique. The results of this assay are very often expressed as optical density at wave lengths ranging from 405–492 (depending on the substrate used). The cut-off points for defining positivity of a given sample are chosen as the mean plus three standard deviations of results obtained from clinically healthy individuals or, in some instances, arbitrarily.

It is not clear from many of the reports whether internal standards (e.g., dilutions of pooled leprosy serum) were included in the assay. It is well known that the ELISA technique is sensitive to even slight variations in the assay conditions. The results in the twilight zone between negative and positive are most susceptible to this variation and can be pushed either way.

To minimize and eventually eliminate variability and to make this assay comparable when performed in various parts of the world, a standard ought to be included as an essential part of the assay. This could be prepared from pooled leprosy sera containing high titers of anti-PGL-I antibody. The synthetic disaccharide conjugated to

bovine serum albumin (D-BSA) can be used as the antigen to isolate and purify this antibody on an affinity column. Inclusion of such a standard would enable the results to be expressed in mass units (mg/ml).

Similarly, the results of the two other specific, quantitative diagnostic tests ([2, 3]) can also be expressed in mass units by using known quantities of the respective monoclonal antibodies. Since each of these methods looks at one epitope, the use of such standards is logical since both the test and the standard would follow the same kinetics in the assay.

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Can *Mycobacterium leprae* Enter the Body Through Unbroken Epithelium?*

To the Editor:

Current concepts of the transmission of leprosy emphasize the importance of the nasorespiratory tract as a route of entry of *Mycobacterium leprae*. However others, for example Leiker, believed that leprosy is more likely transmitted via the skin ([3]).

In one of our earlier studies in nude mice, *M. leprae* entered the nasal mucosa through unbroken epithelium ([1]). An experiment was conducted to find out whether *M. leprae* can penetrate unbroken skin.

A fresh suspension of *M. leprae* containing 1.17×10^9 organisms per ml in Hanks' balanced salt solution was prepared. Twelve 6-week-old nude mice were used for the study. Much of the keratin layer was removed from the skin of the dorsal aspect of the right hind foot in all of the 12 mice using 6 to 8 strokes of Scotch tape. The animals were then anesthetized and both hind foot pads were anchored onto a board, taking care to expose all the dorsum of the hind feet for the experiment. Ten microliters containing 1.17×10^7 *M. leprae* were dropped over the skin of the dorsum of both feet so as to cover an area of approximately 5 mm in diameter. The suspension was allowed to dry under a gentle flow of warm air for 20 to 30 min. Once it dried, a drop of 6% gelatin in water was dropped onto the site and allowed to dry for 15 min. The nude mice were sacrificed at 8, 24, 48, 72, 96, and 120 hr after exposure to *M. leprae*, and a 4-mm punch biopsy of the dorsum of both feet was carried out. The tissues were fixed in 10% buffered Formalin for 48 hr and were processed for paraffin sections. Ten serial sections of 5-μm thick were made from each specimen, and these were stained with a modified Fite's stain ([2]). Every field in all of the sections was examined under a light microscope using an oil immersion lens ($\times$1000).

In the right feet of all animals where an attempt was made to remove the keratin layer, one or more layers of keratin still remained in all of the sections. At 8 hr, there

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