

CORRESPONDENCE

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Polymerase Chain Reaction Applied to Biopsies from
Paucibacillary Leprosy

TO THE EDITOR:

In a previous study (2), we applied the polymerase chain reaction (PCR) to mouse foot pad harvests and human skin biopsies from multibacillary (MB) patients. One of the practical applications of the PCR could be the documentation of paucibacillary (PB) leprosy, which is the subject of the present study.

MATERIALS AND METHODS

Ten 5-mm punch skin biopsies were taken from 10 different patients with the clinical diagnosis of paucibacillary (9 cases) or indeterminate leprosy (1 case) at the Institut Marchoux, Bamako, Mali. The biopsies were divided longitudinally into halves; one was fixed in 10% Formalin for histologic diagnosis, the other was placed in a dry container on ice. The two series of biopsies were sent by air to the Leprosy Laboratory of the Institute for Tropical Medicine, Antwerp, Belgium, where they arrived 48 hr later.

The fresh biopsies were weighed, minced with HCl-cleaned sterilized scissors, suspended in 0.5-ml distilled water, and stored at 20°C until PCR processing. The Formalin-fixed specimens were embedded in paraffin, and the sections were stained with the Triff technique (1).

The sample preparation for the PCR consisted of five cycles of freeze-thawing; the PCR was performed as described previously (2). The reaction was done simultaneously in duplicate on each suspension: one tube with, and a second tube without, an internal

control. Furthermore, between each couple of tubes a blank containing distilled water instead of a biopsy suspension was introduced as the internal control. This tube was subjected to exactly the same manipulations as were the biopsies. When PCR inhibitors were detected in the tube containing the internal control, the reaction was repeated with a 1/10 dilution of the sample.

RESULTS AND DISCUSSION

On histopathological examination, two cases were diagnosed as tuberculoid (TT) and seven as borderline tuberculoid (BT) leprosy. In the possible indeterminate case clinically only some perivascular lymphocytic infiltration was seen, and no definite diagnosis of leprosy could be made. Acid-fast bacilli were not found in any of three sections of each case.

As shown in The Table, 3 specimens produced a positive result, 1 was negative and inhibitors were present in 6. When the latter was diluted 10^{-1} , one specimen still contained inhibitors while the five others gave a negative reaction.

It is striking that, with the exception of biopsy number 1, 2 of the 3 positive results were obtained among the three heaviest biopsies. It seems that a positive result can only exceptionally be obtained in biopsies weighing 30 mg or less.

These results confirm our earlier predictions based on the PCR results from MB leprosy biopsies (2), that the technique as presently performed can only detect *My-*

THE TABLE. Results of PCR reactions on PB specimens.

No.	Histo-logical diagnosis	Weight (mg)	Undiluted sus-pension	Sus-pension diluted 1/10
1	TT	40.6	I ^a	—
2	BT	37.4	+	
3	BT	34.6	+	
4	BT	32.2	I	—
5	PI ^b	26.7	I	—
6	BT	20.4	I	—
7	BT	13.6	—	
8	TT	12.3	I	—
9	BT	9.1	I	I
10	BT	8.5	+	

^a I = Inhibitors.

^b PI = Perivascular infiltration; no definite sign of leprosy.

cobacterium leprae DNA in some PB cases, probably those containing the higher number of *M. leprae* still compatible with PB leprosy.

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