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Letter to the Editor

Microheterogeneity within rRNA of *Mycobacterium gordonae*

Walton and Valesco (5) recently described the identification of *Myobacterium gordonae* from culture by the Gen-Probe Rapid Diagnostic System (Gen-Probe, Inc., San Diego, Calif.), in which a ^{125}I-labeled DNA probe complementary to *M. gordonae* rRNA is employed. Although they reported a sensitivity of 98.7% and a specificity of 98.4% for the assay (with 2 consistent negative *M. gordonae* isolates of 159 total), they also noted certain problems with respect to the hybridization incubation temperature, the density of the culture suspension, and proper function of the sonicator. In particular, fluctuations within the hybridization temperature represented a potential technical pitfall, since raising the temperature of hybridization by as little as 2°C above the optimum of 71 ± 1°C reduced the percent hybridization to below the 10% cutoff value. The authors concluded that "in laboratories where the conventional biochemical tests are replaced with the Gen-Probe *M. gordonae* probe, workers who experience negative hybridization values may inadvertently report false-negative results based on probe results alone."

In our laboratory we use direct sequence determination of 16S ribosomal DNA which has been previously amplified by the polymerase chain reaction for fast and accurate identification of mycobacteria from culture (3). We have found that in contrast to other mycobacteria, which show a conservation of the rRNA sequence at the species level (3; unpublished data), *M. gordonae* exhibits ribosomal DNA variation. As shown in Fig. 1, the rRNA sequence variability of *M. gordonae* is within a region which, because of its interspecies sequence variability, is a common target for diagnostic species-specific DNA probes (1, 2). Since the thermal stability of rRNA-DNA hybrids is dependent on a proper homology of complementary target and probe sequences, we wonder whether this genetic microheterogeneity of *M. gordonae* might be responsible for some of the problems encountered by Walton and Valesco.

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     177                                                  275
I    CGAATAGGACCACAGGACACATGTCCTGTGGT / TGATGGCCTACCAA
II   ..............A..............  /  ..............
III  ..............A..............  /  ........C.....
IV   ..G...........A..............  /  ..............
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FIG. 1. 16S rRNA sequences for *M. gordonae*. The microheterogeneity within the rRNA of *M. gordonae* is characterized by four different nucleotide sequences (I to IV) within *Escherichia coli* positions 100 to 300 (sequence I corresponds to the *M. gordonae* ATCC type strain). The region shown corresponds to *E. coli* positions 177 to 275. Only the relevant parts of the sequence are given, and the nucleotides are numbered according to their respective *E. coli* position. The investigated isolates were also identified by using conventional methods (4).

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Author's Reply

We appreciate the interest of Drs. Kirschner and Böttger. The polymerase chain reaction (PCR) technique for amplification of specific nucleotide sequences holds much promise (2–4). The Rapid Diagnostic System kit (Gen-Probe, Inc., San Diego, Calif.) incorporates an oligonucleotide probe complementary to selected sequences in rRNA. Although this approach is based upon the highly conserved 16S and 23S rRNA molecules, there are regions of high variation between species (1). The work described in the letter of Drs. Kirschner and Böttger suggests unusual rRNA sequence variability of *M. gordonae*. We do not argue the point; indeed, our work concerned itself with the performance of the probe and with its application in the mycobacteriology laboratory (5) rather than with the construction and molecular analysis of the probe. Therefore, we feel a response to the interesting question of genetic microheterogeneity is inappropriate.

The temperature of hybridization is crucial. Generally speaking, when the temperature is substantially below the optimum, distantly related sequences can reassociate, and higher temperatures may cause false-negative hybridization values. Our work supports this observation.

Finally, we feel that out-of-range hybridization temperatures, insufficient density of the test suspension, excessive holding time, and improper functioning of equipment may contribute singly or in tandem toward adverse hybridization values.

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