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Application of Spoligotyping to Noncultured *Mycobacterium tuberculosis* Bacteria Requires an Optimized Approach

In a recent issue we read with interest the contribution of Zink et al. (7) on the characterization of *Mycobacterium tuberculosis* complex DNA in Egyptian mummies by spoligotyping (spacer oligotyping).

Compared with other PCR-based methods that combine detection and typing of such DNA, spoligotyping is more sensitive, because it targets the direct repeats (DRs) present in multiple (sometimes up to 60) copies in the genomic DR locus of *M. tuberculosis* complex bacteria. The well-conserved 36-bp DRs are interspersed with nonrepetitive spacer sequences of 34 to 41 bp in length (2, 3, 4). The variation in the presence of these spacer sequences among strains of the *M. tuberculosis* complex allows for the genotyping of mycobacterial DNA directly isolated from clinical samples without the need to culture these bacteria.

Kamerbeek et al. described spoligotyping for use with purified DNA from cultured strains (2). However, when this method was used to target extracted DNA from *M. tuberculosis* complex bacteria in clinical samples, some of the spacers showed weak hybridization signals. We detected no hybridization signals with a few clinical samples. This is proven by the comparison of the spoligotyping patterns produced from bacteria on a Ziehl-Neelsen (ZN) slide (in dilutions of 1:2, 1:4, and 1:8) with those seen with the method described by van der Zanden et al. (3) (see Fig. 1). Therefore, we optimized our PCR mixture for direct spoligotyping of *M. tuberculosis* complex DNA in clinical samples by using a concentration of 3.0 mM instead of 0.7 mM $MgCl_2$, 15 mM instead of 5 mM Tris-HCl (pH 9.0), and 20 to 50 pmol of primer in PCR. The use of this adjusted protocol yielded a complete spoligotyping pattern for bacteria in clinical samples as compared to that seen with cultured bacteria (3).

We also successfully used the optimized spoligotyping on bacteria from paraffin wax-embedded tissues (4, 5, 6) and from the mummified remains of humans found in an 18th century Hungarian crypt (1). To our surprise, in the current study of Zink et al. (7) the nonoptimized protocol of Kamerbeek et al. (2) was applied to study samples from Egyptian mummies without any modification of the PCR reagents. Hence, in the spoligotyping patterns no hybridization with spacers 2, 14, and 39 was found. We therefore again wish to emphasize that for a reliable and reproducible application of spoligotyping to *M. tuberculosis* complex bacteria in clinical samples, the optimized protocol (1, 3) should be used.

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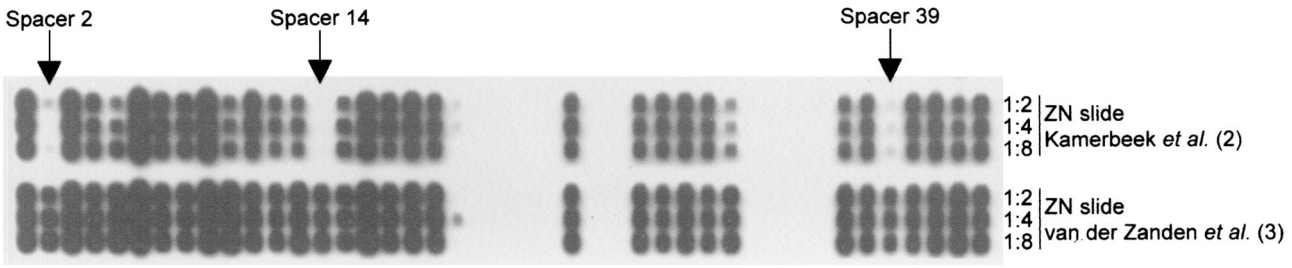

FIG. 1. Comparison of spoligotyping patterns after the application of spoligotyping directly to ZN-stained slides by using the protocols of Kamerbeek et al. (2) and van der Zanden et al. (3). The extracted DNA of the ZN slides was used in the dilutions 1:2, 1:4, and 1:8 in the spoligotyping.

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