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IS*6110*-Based PCR Methods for Detection of *Mycobacterium tuberculosis*

Kent et al. (3) detected *Mycobacterium tuberculosis* by a nested PCR test that amplified a 181-bp target located in insertion sequence IS*6110*, but they also encountered many false-positive results when the test was applied to isolates of mycobacteria that do not belong to the *M. tuberculosis* complex. Because the investigators attributed the false positives to homology between IS*6110* and genomic DNA of the other mycobacterial species, they warned that false-positive PCR results could be a problem with some IS*6110*-based methods for detection of *M. tuberculosis* complex.

Our recent experience in using IS*6110*-based amplifications for detection of *M. tuberculosis* complex is informative on this issue. We selected two such amplifications that others had developed, with respective 123-bp (2) and 317-bp (1, 4) targets, and after slightly modifying the procedures incorporated them into a dual-PCR clinical protocol that we applied to mycobacterial colonies grown on Löwenstein-Jensen or Middlebrook 7H11 medium. The modifications that we made included use of dUTP (rather than dTTP) in both reaction mixes and use of a cycling program that was the same for both amplfications (30 cycles; cycle 1: 94°C for 5 min, 68°C for 45 s, 72°C for 90 s; cycles 2 through 30: 94°C for 45 s, 68°C for 45 s, 72°C for 90 s). The two amplifications were performed in separate tubes in a single thermal-cycler run, and the two amplified products were then electrophoresed in separate lanes of the same ethidium bromide-stained 2% agarose gel. In initial validation studies and in subsequent routine clinical examinations, we used this dual-PCR procedure in blindly testing 116 mycobacterial isolates, all of which were also identified to the species (or complex) level by the Mycobacteriology Laboratory of the New Jersey State Department of Health on the basis of their colony characteristics, chemical reactions, patterns shown on high-pressure liquid chromatography, and DNA-rRNA hybridizations.

The results produced by the dual-PCR procedure and the final identifications by the state laboratory were in total agreement for all 116 isolates: they were positive for *M. tuberculosis* complex in 46 cases and negative in the remaining 70. Identifications made by the state laboratory on the 70 isolates negative for *M. tuberculosis* complex were as follows: *M. avium-M. intracellulare* complex, 50; *M. xenopi*, 7; *M. gordonae*, 7; *M. fortuitum*, 5; and *M. kansasii*, 1. (In the entire series of 116 cultures, there was a single specimen for which the initial PCR results were discrepant, i.e., the 123-bp target was amplified but the 317-bp target was not. An advantage of the dual-PCR approach was demonstrated in th is case, in that duplicate repeats with the two sets of applicable primer pairs produced amplification of both targets, thus identifying the culture as positive for *M. tuberculosis* complex. This diagnosis was later confirmed by the state laboratory.)

Kent et al.'s call for careful validation of any IS*6110*-based method proposed for clinical detection of *M. tuberculosis* complex is surely good advice, but we have not encountered the problem of false-positive results with the two IS*6110*-based tests that we have applied to mycobacterial cultures.

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Authors' Reply

The data presented by Mulcahy et al. confirm the specificity of the PCR methods reported previously (1, 2, 6). One of these PCRs amplifies a region of IS*6110* from 3 to 126 bp, and false-positive results were found with an isolate of *M. simiae*, but none were found with 4 *M. kansasii* and 21 *M. avium* complex strains and single strains of *M. fortuitum*, *M. chelonei*, and *M. gordonae* (2). The other PCR amplifies bases 638 to 954 to yield a 317-bp fragment (6), and the only product obtained among non-*M. tuberculosis* isolates was found in *M. xenopi* and was not confirmed by Southern hybridization. A later large-scale assessment of this method in clinical practice found a 1% false-positive rate (1). Included among these false positives were three patients from whom non-*M. tuberculosis* mycobacteria were isolated on more than one occasion. As both of these PCRs amplify regions of IS*6110* outside the central 181 bp for which we have demonstrated homology, the excellent specificity reported is expected.

The 181-bp region we described is homologous with IS*3* insertion sequences from other genera (4), and using our PCR we have successfully amplified DNA from nonmycobacterial species, including oral bacteria and *Streptococcus pyogenes* yielding a fragment which hybridizes with the 181-bp probe (5).

The use of an insertion sequence (IS) as a PCR target is illogical, as by definition the IS is mobile and may be capable of spreading among other species and genera. As IS*6110* is an IS*3*-like IS, homology and cross-hybridization with IS in other organisms are inevitable. This is of particular importance when specimens which contain a complex flora, such as sputum, are examined, and surprising shared antigens may be found (3). There are little positive data to support the often-stated dogma that IS*6110* is specific to *M. tuberculosis* (7). By diligent design and validation, the authors have avoided the difficulties of an IS*6110*-based method. Despite this, when the clinical significance of a positive result for a patient is so great, a positive IS*6110*-based test should be confirmed by another method.

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