RESEARCH NOTE

Polimerase Chain Reaction for the Detection of *Mycobacterium tuberculosis* Complex

Raúl Diaz, Ernesto Montoro, Jorge L Maestre, Miguel Echemendia, Jose A Valdivia

Instituto de Medicina Tropical "Pedro Kourí" (IPK), Laboratorio Nacional de Referencia de Tuberculosis y Mycobacteria, Apdo 601, Marianao 13, Ciudad de la Habana, Cuba

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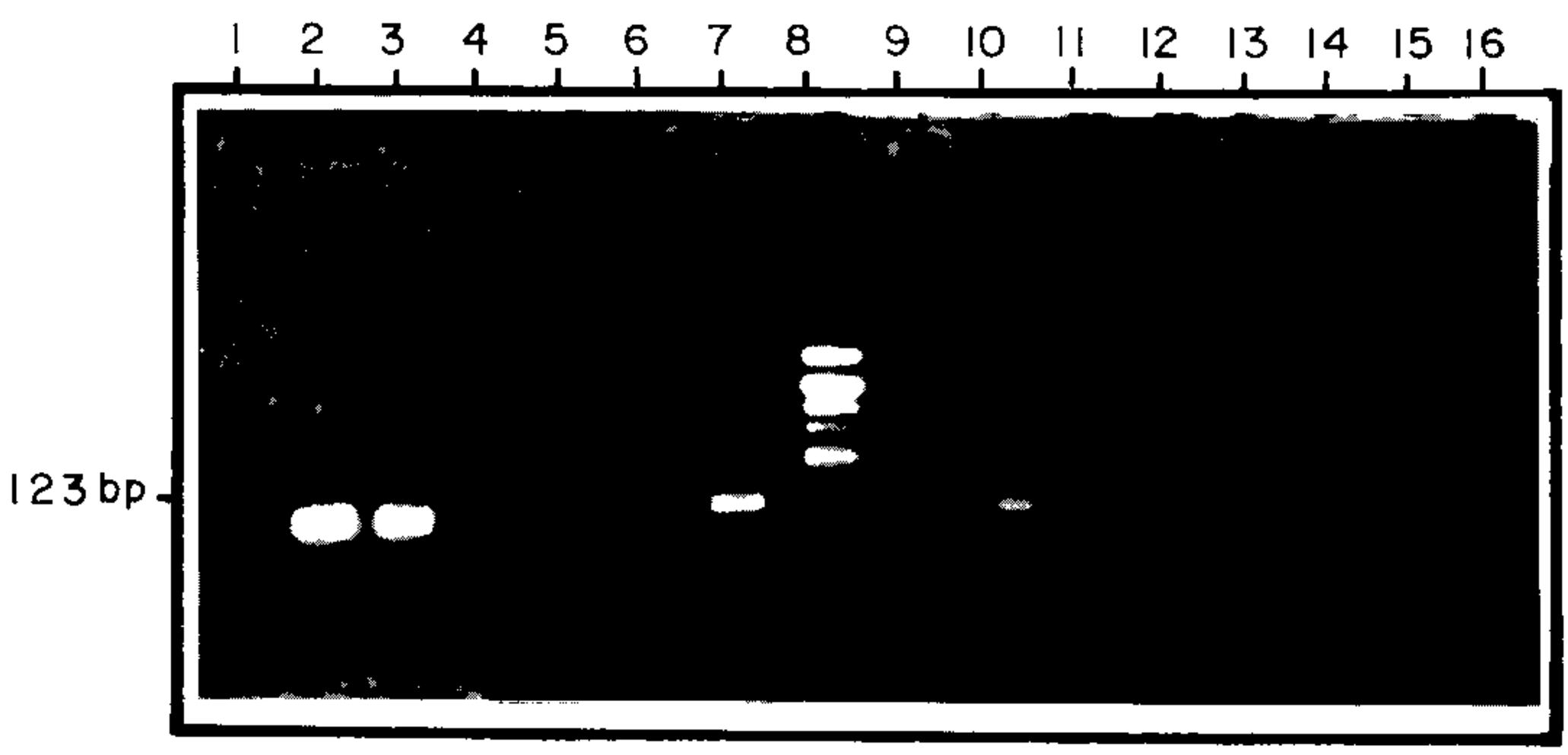
Recent advances in DNA techniques have opened new possibilities to develop a rapid, specific and sensitive method for diagnosis of tuberculosis. Amplification of mycobacterial DNA using the polymerase chain reaction (PCR) has shown that it could be a powerful tool for the identification of *Mycobacterium tuberculosis* in clinical samples (B Savic et al. 1992 *J Infect Dis 166*: 1177-1180).

We applied the PCR technique for the amplification of a 123 base pair (bp) DNA fragment located within insertion element IS6110, which is specific for the *M. tuberculosis* complex as previously reported by D Thierry et al. (1990 *J Clin Microbiol 28*: 2668-2683). Two primers TB-1 (CCTGCGAGCGTAGGCGTCGG) and TB-2 (CTGGTCCAGCGCCGCTTCGG) were used in the amplification reaction as formerly described by KD Eisenach et al. (1990 *J Infect Dis 161*: 977-982). Suspensions of 10^6 bacterial cells from each strain were heated at 95°C for 10 min and used in the PCR. DNA amplifications were done in 100 µl reaction mixtures containing: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.4 mM dNTP, 15 pmoles primers, 10 µl target sample, 2.5 µ Taq polymerase. The reactions were run for 30 cycles: 95°C for 1 min, 68°C for 2 min and 72°C for 1 min using a Gene ATAQ Controller (Pharmacia). A 10 µl aliquot was analyzed by agarose gel electrophoresis, stained with ethidium bromide and visualized by UV transillumination.



Specificity of the amplified fragment using DNA from different mycobacterial strains. Agarose gel electrophoresis containing 1/10 volume of the PCR mixture. Lane 1, no DNA; lane 2, *Mycobacterium tuberculosis* H37Rv; lane 3, *M. tuberculosis* H37Ra; lane 4, *M. habana* (220 strains); lane 5, *M. habana* (337 strains); lane 6, *M. kansasii;* lane 7, *M. bovis* BCG; lane 8, Alu I-digested pBR 322 plasmid used as DNA size marker; lane 9-11, *M. tuberculosis* strains isolated from Cuban patients; lane 12, *M. simiae* (serotype 1); lane 13, *M. intracellulare*; lane 14, *M. scrofulaceum*; lane 15, *M. gordonae;* lane 16, *M. phlei.*

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We used different mycobacterial strains: 23 *M. tuberculosis* complex strains (*M. tuberculosis* H37Rv and H37Ra, *M. bovis* BCG and 20 *M. tuberculosis* strains isolated from Cuban patients) and 13 mycobacteria other than tuberculosis (MOTT) strains.

All of the 23 strains of the *M. tuberculosis* complex tested, amplified the expected fragment (123 bp). It was not necessary to use purified DNA to obtain the amplified fragment. No detectable DNA product was observed from 13 MOTT strains (Fig.). These results were similar to other previously reported by Eisenach et al. (*loc. cit.*) and GE Buck et al. (1992 *J Clin Microbiol 30*: 1331-1334).

The sensitivity of the technique was determined using 10 fold serial dilutions from a heated suspension of *M. tuberculosis* H37Rv. Our results indicated that at least 10 mycobacterial cells can be detected without any hybridization method coupled (data not shown). Kocagöz et al. (1993 *J Clin Microbiol 31*: 1435-1438) used a similar heating procedure to prepare the samples for the amplification and they also determined that 10 microorganisms could be detected directly in agarose gel.

This stuy demonstrated the utility of the IS6110 as a very specific target sequence in the diagnosis of tuberculosis by DNA amplification and how this element is repeated several times in the *M. tuberculosis* chromosome a good sensitivity can be achieved. Preparing samples by heating confirm that this simple method is capable of releasing DNA for the PCR and it can be used in the routine processing of clinical specimens. Now, we are starting to apply this technique in the direct detection of *M. tuberculosis* in clinical samples.