Real-time PCR optimization to identify Mycobacterium tuberculosis complex strains in clinical samples.

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During recent years several molecular techniques have become available for *Mycobacterium tuberculosis* complex (MTC) detection, both for clinical samples and for isolates. One of the techniques more widely used is real time PCR in combination with nucleic acid amplification protocols. There are numerous studies based on PCR for the diagnosis of tuberculosis although the different protocols and primers used in the laboratory, together with the variability in the diagnostic performance of the methods tested, require that a comparative study be performed. Furthermore, the fact that the detection from clinical samples requires using highly sensitive targets suggests that this type of study should include multicopy targets to compare their efficiency with respect to the single copy. Our aim was to identify the members of the MTC using real-time PCR assays based on SYBR Green, among a large panel of isolated bacterial strains and clinical samples. We chose three targets (IS6110, senx3-regx3 and cfp32) and the optimal values for each PCR assay were empirically defined by testing in triplicate different concentrations of $MgCl_2$ and primer sets and different annealing temperatures. These conditions were determined based on the specific amplification reactions that showed a lower Ct value, higher fluorescence and absence of non-specific PCR products. The analytical sensitivity was evaluated by ten-fold serial dilutions of DNA from MTC and the specificity was tested by 62 different microorganisms, including bacteria related with the MTC. The diagnostic yield was evaluated in 66 specimens from patients with suspected tuberculosis; 30 had tuberculosis and 36 (control group) had different diseases. Under the conditions that resulted in optimization, standard curves showed that senx3-regx3 assay was the most efficient, followed by IS6110 and cfp32. However, the detection of bacterial DNA was faster with the repetitive element IS6110, with Ct values of up to 3 and 9 cycles of difference with respect to senx3-regx3 and cfp32. The analytical specificity, done only with the senx3-regx3 and IS6110 targets, was in the order of 100 and 93.5%, since IS6110 amplified various non-tuberculous micobacteria. For all the clinical samples studied, the sensitivity of both assays was identical (93.3%) but the specificity of senx3-regx3 (100%) was higher than that of IS6110 (94.7%). In conclusion, real time PCR assay-SYBR Green based on the targets senx3-regx3 is highly reproducible and more sensitive and specific than the assays based on IS6110 or cfp32. The protocol developed in this study provides an appropriate and rapid tool to identify the strains of MTC in different clinical isolates and specimens.