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Quantitative polymerase chain reaction analysis of pathogenic DNA sequence using an internal DNA sequence standard constructed by recombinant DNA methodology

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The successful development of sensitive and reliable quantitative assays for polymerase chain reaction (PCR) products using High Performance Liquid Chromatography (HPLC) have opened up new research and clinical applications that promote the understanding of pathology, pharmaco-toxicology, therapeutic management and diagnosis of various diseases. However, it has been difficult to correlate the amount of PCR products output to the amount of DNA targets input which should be the more meaningful data for clinical applications. There are multiple factors leading to this analytical problem, such as the reproducibility of PCR thermocycling runs, the integrity of reagents used, the PCR conditions and kinetics, the sample preparation methodologies and matrix interference, etc. By constructing a recombinant cytomegalovirus (CMV) control target plasmid which contained a modified CMV AD169 sequence that shared the PCR primer sequence of native CMV AD169 and co-amplified both the unknown and control plasmid targets, we were able to by-pass some of the kinetic PCR problems and to quantify native CMV AD169 targets. PCR co-amplification of the CMV control and CMV native plasmid targets resulted in two distinctively separable PCR products of 362 bp and 152 bp respectively, with a 15 min HPLC run. During PCR co-amplification there was an overall decrease as well as competition in the synthesis of the PCR products from both plasmid targets as compared to non-co-amplified co[illegible]itions, but there existed a linear relationship between th[illegible] molar ratio of input targets and the molar ratio of their corresponding output products. Using this mathematical approach, we have achieved very good accuracy (recovery %) of 100 $\pm$ 16% and within-run imprecision (CV) of 12% for a quantitative PCR assay of input native CMV AD169 DNA target sequence over a 5 log range (0.0031 to 31 amoles, corresponding to 2×10^3 - 2×10^7 copies of DNA target sequence). Improvement in sample preparation and optimization of primer-target sequences are necessary before this methodology can be applied clinically. This quantitative PCR detection method provides an analytical model to refine PCR-based techniques.