OP 2

Development and validation of an in house multiplex real time PCR for quantification of all four serotypes of dengue virus

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Objectives: In order to determine the infecting dengue virus (DENV) serotype and also to quantify the amount of virus in clinical samples and cell cultures, we proceeded to optimize and validate a quantitative real time RT-PCR assay.

Methods: WHO reference strains of the four DENV serotypes were cultured on C6/36 cell lines for 7 days. The supernatants were used to determine plaque forming units (pfus) using BHK cell lines, which is indicative of infecting virus particles. cDNA was synthesized from RNA extracted from the virus culture supernatants. A tenfold dilution series (106 to 101 pfu/ml) of the known pfus of the viruses was prepared using the cDNA for standard curves. Data were analyzed using applied biosystems sequence detection systems. The threshold cycle value (Ct) for each reaction was determined by manually setting the threshold limit. Each standard curve was initially generated individually and later was used in a multiplex assay. All experiments were done in triplicate.

Results: The optimized multiplex method detected all four DENV serotypes and generated standard curves with correlation coefficients (R2 values) above 0.95, which suggested that the standard curves and dilutions were of acceptable quality. The slope values of the standard curves were between -3.1 and -3.8 for all assays, implying that the PCR reactions were quite efficient.

Conclusions: We have optimized and validated a multiplex quantitative real time RT-PCR assay, which can be effectively used to determine the infecting DENV in samples and also quantify the amount of virus.