Development of a method to control the RNA extraction and reverse transcription steps for the detection of dengue virus present in human blood samples

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Resumen

Aims: Molecular biology techniques based on the detection of genomic sequences by reverse transcription combined with polymerase chain reaction (PCR) have enabled the detection of different RNA viruses in serum or plasma samples. Since the dengue epidemic outbreak declared in Argentina in 2009, numerous patients' samples were analyzed for the acute phase of infection. One of the main methodological drawbacks is the lack of internal control to measure the effectiveness of the viral extraction and reverse transcription process. In this article, we propose to standardize a molecular method to detect beta actin (β-Act) and glucose 6 phosphate dehydrogenase (G6PDH) complementary DNAs (cDNAs) present in patient's plasma/serum, as a control process. Results: RNA extraction, reverse transcription, and PCRs for human G6PDH, β-Act, and the dengue virus genome were performed. cDNA fragments for β-Act and G6PDH were amplified for all samples, regardless of the presence or absence of viral RNA. Conclusions: Amplification of β-Act and G6PDH cDNAs can be used as a control for the extraction and reverse transcription processes during dengue virus detection. This could also be a useful method for controlling the above steps when infections caused by other RNA viruses are studied, even if another methodology is employed, such as real-time PCR.

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