RNA INTEGRITY (RIN) NUMBER AND THE INFLUENCE ON CYCLE THRESHOLD (CT) VALUE IN A REAL-TIME QRT-PCR

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The quality of RNA after extraction plays a major role in the success of downstream molecular biology applications such as quantitative reverse-transcriptase PCR (qRT-PCR) or micro-arrays analysis. Although there is no gold standard to determine RNA quality, microcapillary electrophoresis systems are becoming very popular, and are on their way to becoming the standard in RNA quality assessment. An example of these microcapillary systems, is the Agilent 2100 Bioanalyzer automated microcapillary electrophoresis system that provides an effective method to determine RNA quality by generating a RNA integrity number (RIN number).

The major objective of this study was to determine whether there is any correlation between RIN numbers and cycle threshold (Ct) values in a qRT-PCR. For this purpose, we made six dilutions of commercially available intact total human RNA (Hmn Hela-S3, Ambion): 200, 100, 50, 25, 12.5 and 6.25 ng/µl. We used heat to degrade these RNA samples in order to generate three main RIN values for any given RNA concentration. For each RNA concentration, the effect of RNA degradation on qRT-PCR was investigated by correlating RIN values with the Ct values. The expression levels of GAPDH gene was assessed on the specimens using real time qRT-PCR. We found that with high RNA quality (high RIN values) and high RNA concentration (>50 ng/µl) there was good correlation between RIN numbers and Ct values. The Ct values decreased with the increased RIN values. However, this trend was not consistent when lower RNA concentration (<50 ng/µl) were investigated.

These results suggest that for partially degraded and lower RNA concentration, the Agilent 2100 Bioanalyzer overestimated the RIN values. This is particularly true for forensic stain samples that are usually at lower RNA concentration and highly degraded.