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IMPROVED METHODOLOGY FOR ASSESSMENT OF mRNA LEVELS IN BLOOD OF PATIENTS WITH *FMR1* RELATED DISORDERS.

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Elevated levels of *FMR1* mRNA in blood have been implicated in RNA toxicity associated with a number of clinical conditions. Due to the extensive inter-sample variation in the time lapse between the blood collection and RNA extraction in clinical practice, the resulting variation in mRNA quality significantly confounds mRNA analysis by real-time PCR. Here, we developed an improved method to normalize for mRNA degradation in a sample set with large variation in rRNA quality, without sample omission. Initially, RNA samples were artificially degraded, and analyzed using capillary electrophoresis and real-time PCR standard curve method. We found that although several chromatographic features were reliable predictors of total RNA degradation, their use for target gene normalization was inferior to internal control genes, of which *GUS* was the most appropriate. Using *GUS* for normalization, we examined in the whole blood the relationship between the *FMR1* mRNA and CGG expansion in a non-coding portion of this gene, in a sample set (n=30) with the large variation in rRNA quality. By combining *FMR1* 3' and 5' mRNA analyses the confounding impact of mRNA degradation on the correlation between *FMR1* expression and CGG size was minimized, and the biological significance increased from p=0.046 for the 5' *FMR1* assay, to p=0.018 for the combined *FMR1* 3' and 5' mRNA analysis. In conclusion, our observations demonstrate that through the use of an appropriate internal control and the direct analysis of multiple sites of target mRNA, samples that do not conform to the conventional rRNA criteria can still be utilized to obtain biologically/clinically relevant data. Although, this strategy clearly has application for improved assessment of *FMR1* mRNA toxicity in blood, it may also have more general implications for gene expression studies in fresh and archival tissues

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