Analysis of miRNA in blood plasma – challenges and solutions

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Background:

Micro RNA (miRNA) are small, single-stranded non-coding RNAs consisting of 20-22 nucleotides that regulate the gene expression. Their expression level and type have been associated with early diagnosis, targeted therapy and prognosis of various diseases. Therefore, miRNA are potential biomarkers and it is of high importance to have reliable methods for miRNA detection.

The most common method for sensitive and specific analysis of miRNA form biological fluids such as plasma and serum is quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Although numerous studies have evaluated extraction efficiency and normalization of the extracted sample, there is still a great variability in results among laboratories.

In order to obtain accurate miRNA measurement, high quality and high yield sample because of the low concentration of miRNA in plasma is required. Factors mostly influencing purity are sample matrix, extraction residual reagents, plasma sample storage conditions and some preanalytical factors such as hemolysis. Various extraction kits and modifications of extraction processes have been investigated and will be presented.

Another important issue is variation in qRT-PCR analysis which can be mitigated by normalization. We will discuss differences between global normalization, use of endogenous reference gene and external references.qRT-PCR as any other PCR is influenced by amplification efficiency. There are protocols based on amplification efficiency which can help scientists to make a correction of annealing and primer conditions in order to ensure accurate analysis.

Conclusion:

To be able to transfer analysis of circulating miRNA from plasma into validated biomarker-based test for routine clinical use, it is necessary that all preanalytical, analytical and postanalytical factors are considered and evaluated.