miRiam – NEW TRENDS IN microRNA QUANTIFICATION

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Introduction

MicroRNA (miRNA) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target miRNAs. They are involved in a wide range of biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation and also in pathophysiology of many diseases. Recently, numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of cardiovascular diseases, neurological disorders, cancer, metabolic syndromes and many other diseases. Monitoring the level of specific miRNA together with protein-based biomarkers may represent more efficient tool for diagnosis of these diseases and prognosis estimation. Current methods of miRNA determination are either too low in specificity and sensitivity or very expensive and high-technology demanding like Next-generation sequencing (NGS) or qRT-PCR which is considered to be the gold standard for miRNA expression analysis.

We are introducing a novel, immunoassay-based method of miRNA quantification which involves hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/RNA hybrids are then transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. After washing, the solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized (after another washing step) by a chromogenic substrate.

Our immunoassay exhibits superior analytical characteristics and strong correlation with qRT-PCR (R/two.superior = 0.95687). We found a strong correlation between qRT-PCR and miREIA-21-5p, miREIA assays exhibits excellent analytical characteristics and strong correlation with qRT-PCR.

miRNA quantification

E) Comparison with qRT-PCR

Five different miRNAs (miR-122-5p; miR-21-5p; miR-191-5p; miR-223-3p and miR-16-5p) isolated from whole blood were quantified using LNA nucleotide probes from Exiqon.

We compared the total time needed for miRNA quantification by two different methods: qRT-PCR and miREIA. The total assay time of miREIA is significantly shorter when compared to qRT-PCR.

References

11. Kappel, Andreas, et al. “miRNA – miRNA enzymes immunoassay (B) The hybridization mixture is transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched RNA/DNA-biotin hybrids. In the next step, the solid phase is washed and subsequently incubated with streptavidin-HRP conjugate. Finally, the resulting complexes are visualized by chromogenic substrates 3,3’,5,5’-tetramethylbenzidine (TMB).