**MicroRNA-BASED DIAGNOSTICS IN CARDIOVASCULAR DISEASE**

**Introduction**

MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target mRNAs. 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 many diseases.

MicroRNA-21 (miR-21) is highly expressed in the cardiovascular system. Recent studies have revealed that its expression is dysregulated in heart under cardiovascular disease conditions such as proliferative vascular disease, cardiac hypertrophy and heart failure or ischemic heart disease. miR-21 has been found to play important roles in vascular smooth muscle cell proliferation and apoptosis, cardiac cell growth and death and cardiac fibroblast functions.

**Method**

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 specificity, limit of detection as low as 0.1 attomol/µl miRNA, excellent analytical characteristics and strong correlation with the qRT-PCR method (Pearson correlation coefficient > 0.9).

Moreover, the assay can be run on common immunoassay analyzers, is compatible with standard clinical workflow, does not require amplification steps and results are obtained in less than three hours including miRNA profiling. Our method enables to analyze miRNA using conventional immunoassay analyzers, thus, it can promote utilization of miRNA biomarkers in clinical and laboratory practice.

**References:**

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**Conclusion**

- We are introducing a novel method for miRNA quantification.
- The miREIA-21-5p assay exhibits excellent analytical characteristics and strong correlation with qRT-PCR.
- We compared the total time needed for miRNA quantification by two different methods: qRT-PCR and miREIA-21-5p. The total assay time of miREIA-21-5p is significantly shorter when compared to qRT-PCR.