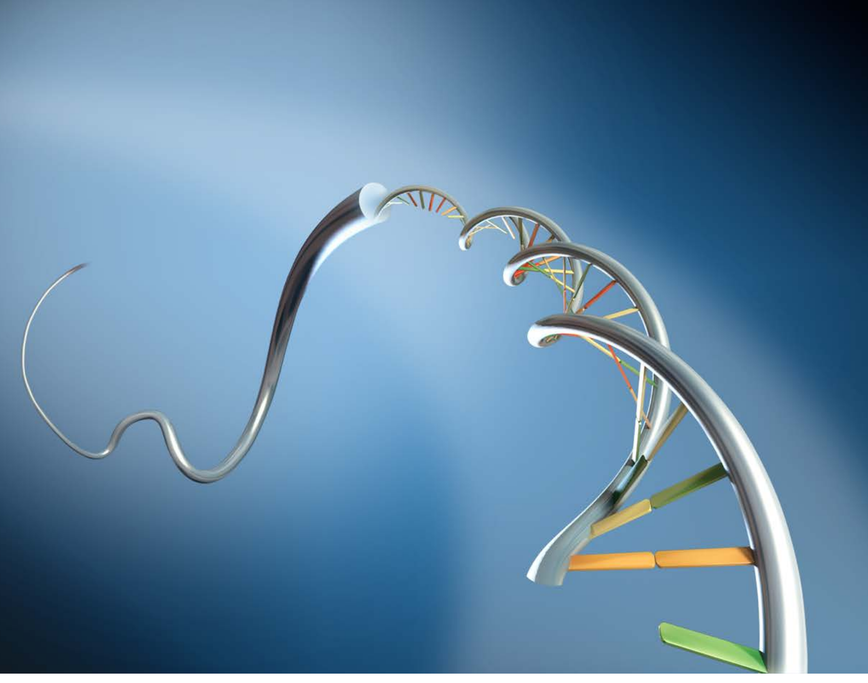
miRiam – NEW TRENDS IN microRNA QUANTIFICATION



J. Jancovicova[1], M. Hlozankova[1], B. Dvorakova[1], M. Buresova[1], M. Hamanova[1], L. Chalupova[1], K. Cuchnova[1], A. Kappel[2], A. Keller[3], V. Ruzicka[1]

1 BioVendor-Laboratorní medicína a.s., Research & Diagnostic Division, Brno, Czech Republic
2 Corporate Technology, Siemens AG, Erlangen, Germany
3 Clinical Bioinformatics, Medical Faculty, Saarland University, Germany

Introduction

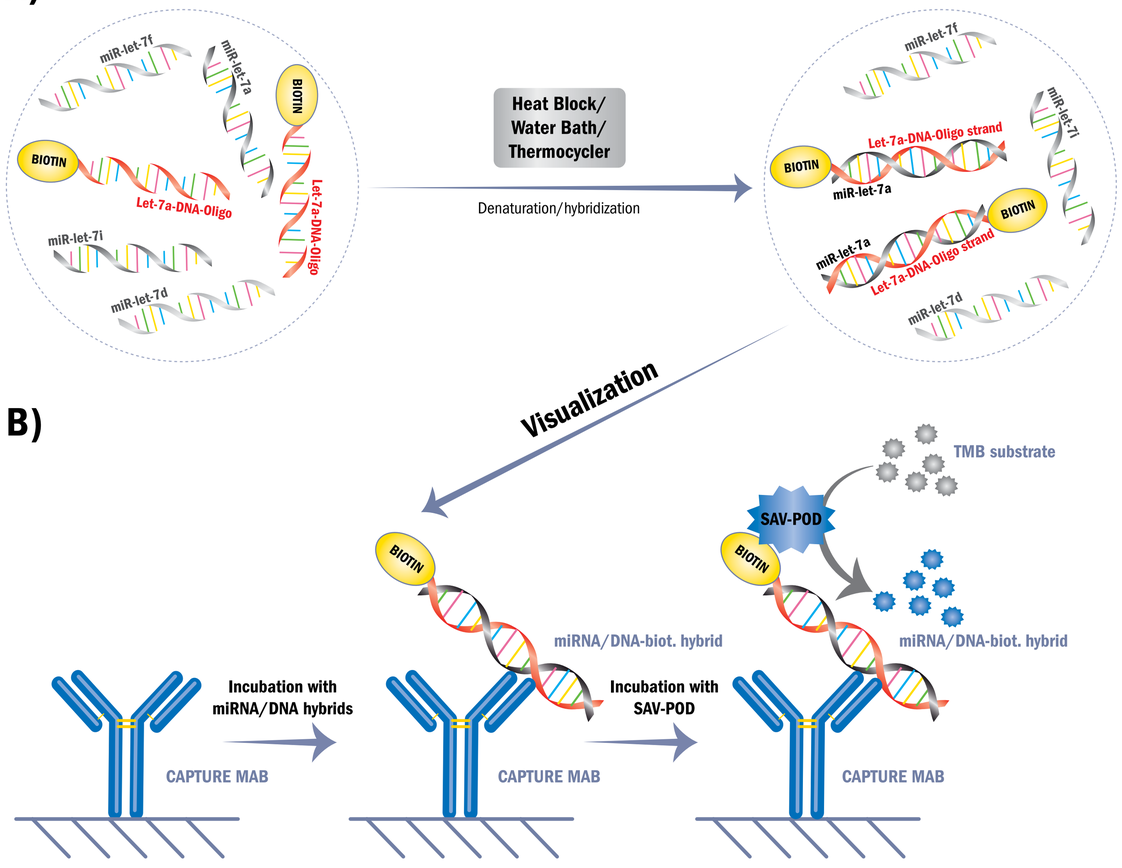
MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target miRNAs. They are involved in a wide range of biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or 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 syndrome 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 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.

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.

miRNA quantification

A)

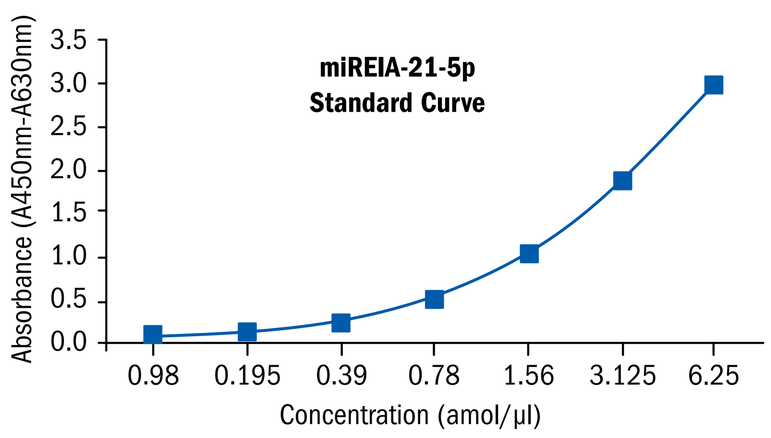


Hybridization of miRNA to complementary biotinylated DNA (A)
Defined amount of specific biotin-labeled DNA oligonucleotide is hybridized to miRNA isolated from blood sample.

miReia – miRNA enzyme 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 substrate 3,3',5,5'-tetramethylbenzidine (TMB).

miREIA-21-5p

C) Calibration range



The calibration range of the miREIA-21-5p assay is 0.098-6.25 amol/ul.

D) Dilution linearity

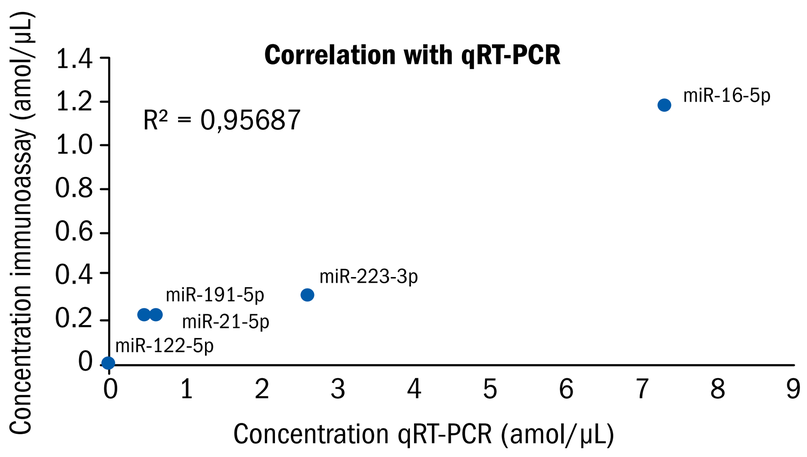
Two microRNA isolates from whole blood samples were serially diluted.

Sample	Sample dilution	Observed concentration amol/µl	Expected concentration amol/µl	Recovery O/E (%)
Sample1	-	10.2	-	-
	2	5.0	5.1	99
	4	2.7	2.6	105
	8	1.3	1.3	100
Sample 2	-	20.4	-	-
	2	10.1	10.2	99
	4	4.8	5.1	94
	8	2.6	2.6	100

Recovery was determined to be 94 – 105%

E) Comparison with qRT-PCR

Five different miRNAs (miR-122-5p; miR-21-5p; miR191-5p; miR-223-3p and miR16-5p) isolated from whole blood were quantified by miREIA and by qRT-PCR using LNA nucleotide probes from Exiqon.



We found a strong correlation between qRT-PCR and miREIA-21-5p, the Pearson correlation coefficient being >0,9.

F) Total Assay Time

LNA qRT-PCR	miReia
Adjust each of the template RNA samples to a optimal concentration (15 min)	Hybridization (10 min)
Reverse transcription (65 min)	Preparation and pipeting of sample to the plate (15 min)
Preparation of samples after reverse transcription and PCR reagents (20 min)	Incubation of hybrids with monoclonal Antibody (60 min)
Preparation the PCR reaction plate (15 min)	Incubation with SAV-POD (30 min)
Run the PCR reaction plate (90 min)	Incubation with substrate (10 min)
Total time to result 3,5 hours	**Total time to result 2 hours**

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.

Conclusion

- We are introducing a novel method for miRNA quantification
- miREIA assays exhibits excellent analytical characteristics and strong correlation with qRT-PCR

References:

1. Shenoy, Archana, and Robert H. Blelloch. *Regulation of microRNA function in somatic stem cell proliferation and differentiation.* Nature Reviews Molecular Cell Biology 15.9 (2014): 565-576.
2. Rottiers, Veerle, and Anders M. Näär. *MicroRNAs in metabolism and metabolic disorders.* Nature reviews Molecular cell biology 13.4 (2012): 239-250.
3. Teruel-Montoya, R., F. R. Rosendaal, and C. Martínez. *MicroRNAs in hemostasis.* Journal of Thrombosis and Haemostasis 13.2 (2015): 170-181.
4. Jovanovic, M., and M. O. Hengartner. *miRNAs and apoptosis: RNAs to die for.* Oncogene 25.46 (2006): 6176-6187.
5. Singh, Ram Pyare, et al. *The role of miRNA in inflammation and autoimmunity.* Autoimmunity reviews 12.12 (2013): 1160-1165.
6. Esquela-Kerscher, Aurora, and Frank J. Slack. *Oncomirs—microRNAs with a role in cancer.* Nature Reviews Cancer 6.4 (2006): 259-269.
7. Romaine, Simon PR, et al. *MicroRNAs in cardiovascular disease: an introduction for clinicians.* Heart 101.12 (2015): 921-928.
8. Tan, Lin, et al. *Genome-wide serum microRNA expression profiling identifies serum biomarkers for Alzheimer's disease.* Journal of Alzheimer's Disease 40.4 (2014): 1017-1027.
9. Wang, Jin, Jinyun Chen, and Subrata Sen. *MicroRNA as biomarkers and diagnostics.* Journal of cellular physiology 231.1 (2016): 25-30.
10. Hayes, Josie, Pier Paolo Peruzzi, and Sean Lawler. *MicroRNAs in cancer: biomarkers, functions and therapy.* Trends in molecular medicine 20.8 (2014): 460-469.
11. Kappel, Andreas, et al. *MicroRNA in vitro diagnostics using immunoassay analyzers.* Clinical chemistry 61.4 (2015): 600-607.

BioVendor
Research
and Diagnostic Products