miRiam – THE NEW INSIGHT INTO miRNA QUANTIFICATION

Jana Jancovicova[1], Andreas Kappel[2], Herbert Schwarz[3], Martina Hlozankova[1], David Stejskal[4,5,6], Viktor Ruzicka[1], Andreas Keller[7]

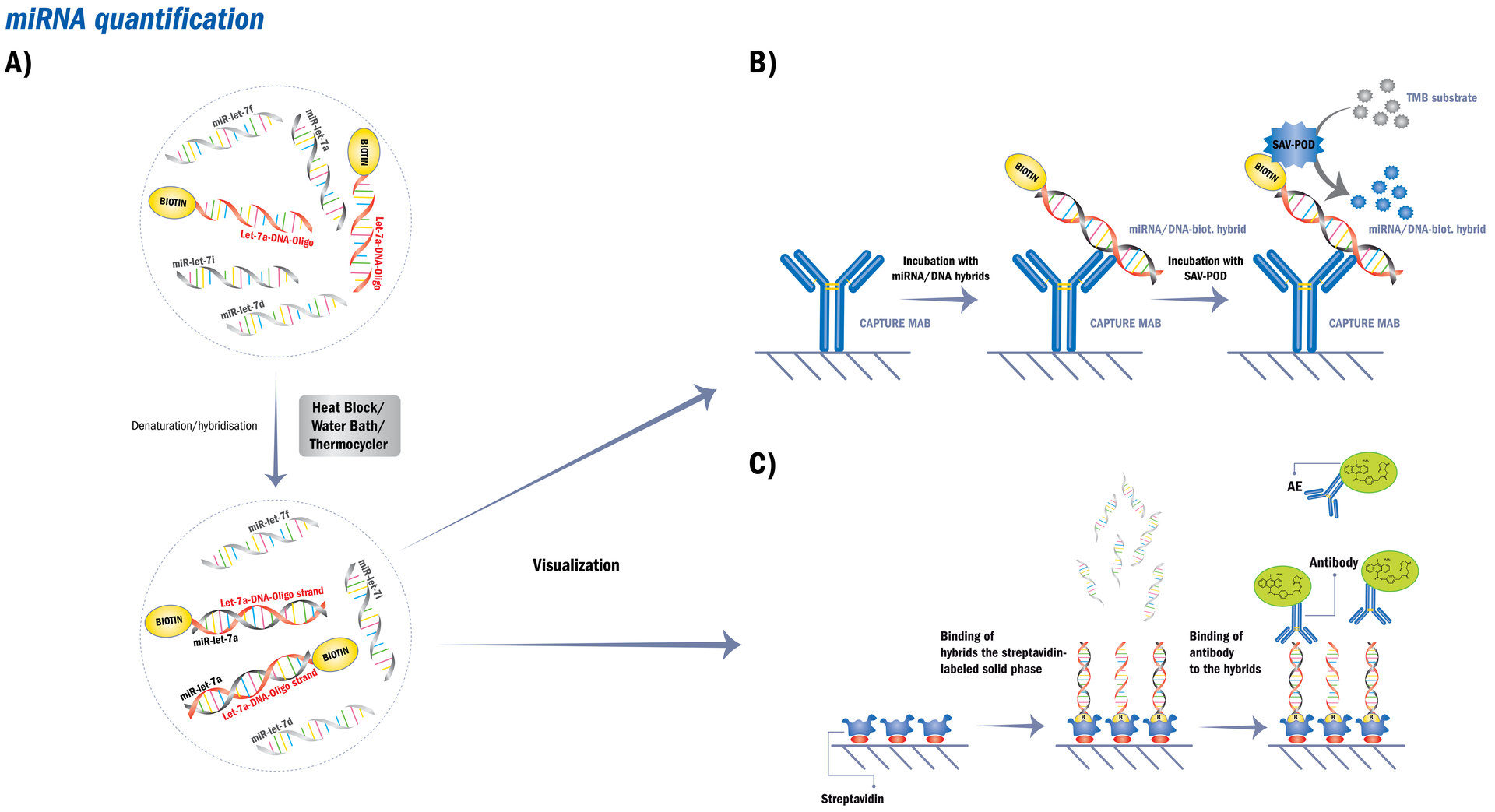
1 BioVendor Laboratorní medicína a.s., Research & Diagnostic Division, Brno, Czech republic · 2 Corporate Technology, Siemens AG, Erlangen, Germany
3 Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany · 4 AGEL Research and Training Institute, Prostejov, Czech Republic
5 Institute of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
6 Department of Laboratory Medicine, Central Moravian Hospital County Inc., Prostejov, Czech Republic · 7 Clinical Bioinformatics, Medical Faculty, Saarland University, Saarbrücken, Germany

Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in the gene translation via silencing or degradation of target mRNAs. They are involved in virtually all physiologic processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation and in pathology of many diseases such as cancer, heart disease and neurological disorders [1-8]. Recently, miRNA has been proposed as a powerful biomarker for the prediction of diseases, treatment response or disease progression [9-11]. Currently used methods for miRNA determination are either low in specificity and sensitivity or there are very expensive and high-technology demanding such as NGS or qRT-PCR which is considered to be a gold standard for miRNA expression analysis.

We are introducing a novel method for quantification of miRNA based on immunoassay. The novel approach involves hybridization of miRNA isolated from patient sample to a complementary biotinylated DNA oligonucleotide probe followed by monoclonal antibody detection of perfectly matched DNA/miRNA hybrids with colorimetric or chemiluminescent visualization. Our immunoassay has an analytical specificity of 99,4%, limit of detection as low as 1 pmol/L miRNA and great correlation with the qRT-PCR method (Pearson correlation >0,994) [11]. Besides outstanding sensitivity and specificity, the assay can be run on standard immunoassay analyzers, the method is compatible with classical clinical workflow, does not require amplification steps and time to result is less than three hours including purification and miRNA profiling. This method provides an opportunity to analyze miRNA using conventionally available immunoassay analyzers and thus speed-up utilization of miRNA biomarkers in clinical and laboratory practice.

miRNA quantification



The first step of miRNA quantification is hybridization of DNA and complementary miRNA (A).
Defined quantity of biotin-labeled specific DNA oligonucleotide is hybridized with isolated miRNA from blood sample under specific conditions which ensure that only a single, complementary miRNA species present in patient sample will hybridize. The quantification of perfectly matched RNA/DNA heterohybrids is performed using solid-phase immunoassay technique with two possible methods of visualization:

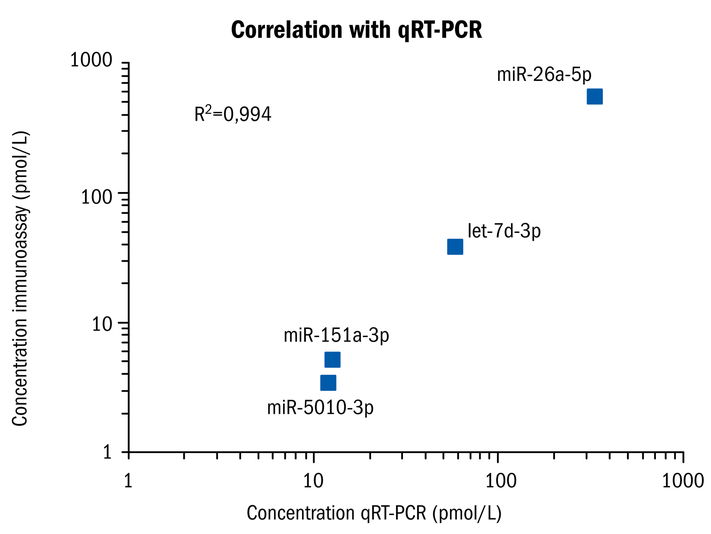
miReia – miRNA **e**nzyme **i**mmuno**a**ssay **(B)**
The hybridization mixture is transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched RNA/DNA-biotin hybrids. In the next step, the solid phase is washed followed by incubation with streptavidin-HRP conjugate. Finally, the reaction is visualized by chromogenic substrate ~ 3,3',5,5'-tetramethylbenzidine (TMB).

miRacle – miRNA **a**ntibody/**c**apture **l**uminom**e**try **(C)**
The streptavidin-coupled magnetic beads are added into the hybridization mixture and the biotinylated DNA catcher binds to the beads through biotin-streptavidin interaction. Unbound miRNAs and other RNAs are washed away so that just the hybrid RNA/DNA duplexes remain. Finally, a monoclonal antibody specific to RNA/DNA hybrids labeled with dimethyl acridinium ester is added and the chemiluminescent signal is induced.

In both visualization approaches the intensity of signal is proportional to the number of RNA/DNA hybrids and/or to the concentration of specific miRNA species present in the blood sample.

Comparision with qRT-PCR

D)



E)

Time to result comparison

TaqMan qRT-PCR	miReia	miRacle
Reverse transcription (65 min)	Hybridization (10 min)	Hybridization (6 min)
Creation and set up a plate document (15 min)	Preparation and pipeting of sample to the plate (15 min)	Incubation of hybrids with solid phase (18 min)
Preparation the PCR reaction plate (15 min)	Incubation of hybrids with monoclonal Antibody (60 min)	Washing (7 min)
Run the PCR reaction plate (90 min)	Incubation with SAV-POD (30 min)	Incubation of hybrids with monoclonal antibody (18 min)
	Incubation with substrate (10 min)	
Total time to result 3 hours	**Total time to result 2 hours**	**Total time to result less than 1 hour**

Correlation with qRT-PCR (D)
Four different miRNAs (miR-5010-3p; miR-151a-3p; miR26a-5p and let-7d-3p) were isolated from whole blood and quantified either using novel miRNA quantification method (miRiam) or by gold standard qRT-PCR using stem-loop primers for qRT-PCR with TaqMan® probes. We found a high correlation between qRT-PCR and the novel miRNA quantification method (miRiam). The Pearson correlation >0,994, P=0,006.

Time to result comparison (E)
We compared time of analysis needed by different methods: qRT-PCR, miRNA quantification with colorimetric detection (miReia) and miRNA quantification with chemiluminescent visualization (miRacle) on the automatic platform. Both manual and automatic miRNA quantification have significantly lower time-to-result when compared to qRT-PCR.

Conclusion

- We are introducing a novel method for miRNA quantification generally named **miRiam – miR**NA **i**mmuno**a**ssay **m**ethod
- The system utilizes combination of DNA/miRNA hybridization followed by two possible methods of visualization
  - Colorimetric - **miReia** - **miR**NA **e**nzyme **i**mmuno**a**ssay
  - Chemiluminescent - **miRacle – miR**NA **a**ntibody/**c**apture **l**uminom**e**try
- The method has great correlation with gold standard technique for miRNA quantification - qRT-PCR (The Pearson correlation >0,994)
- The employment of conventional immunoassay in the miRNA field could speed up the utilization of these biomarkers in clinical and laboratory practice

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