NOVEL IMMUNOASSAY TOOL FOR QUANTIFICATION OF STROKE RELATED miRNA BIOMARKERS

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INTRODUCTION

- miRNAs are promising stroke biomarkers, but methods for their detection have limitations especially reverse transcription step in the most widely used RT-qPCR principle [1].
- We present novel method for quantitative miRNA measurement based on immunoassay – miREIA.
- Analytical characteristics and correlation with RT-qPCR (ID3EAL miRNA qPCR Assays; MIRXES Life Science) were evaluated on 3 stroke related miRNAs hsa-miR-223-3p, hsa-miR-486-5p and hsa-miR-let-7b-5p [2-4].

METHODS

miREIA - an enzyme immunoassay for miRNA quantification involves

- hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA probe (Fig. 1a).
- the DNA/RNA hybrids are transferred into microplate wells pre-coated with mono-clonal antibody specific to perfectly matched DNA/miRNA hybrids (Fig. 1b).
- after washing, the solid phase is incubated with streptavidin-HRP conjugate followed by another washing step. The resulting complexes are visualized by chromogenic substrate (Fig. 1b).
- the absorbance is proportional to the concentration of target miRNA [5].

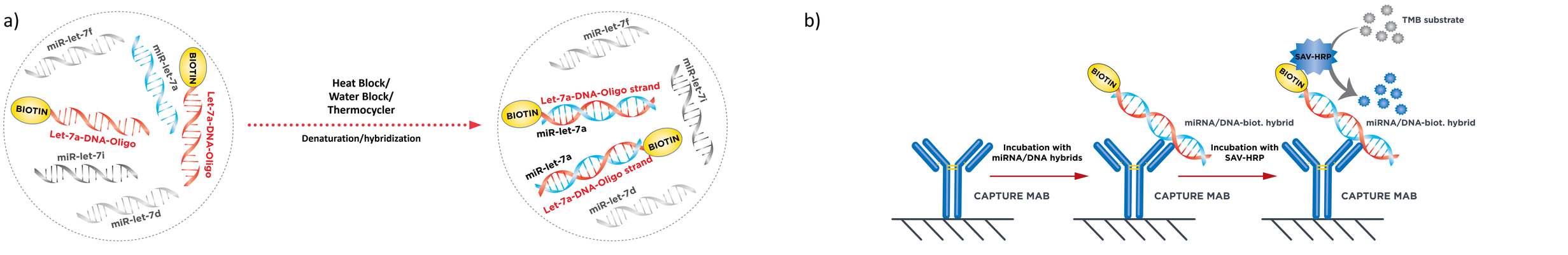


Fig. 1: miREIA workflow (a – hybridization; b – immunoreaction and detection)

RESULTS

miREIA analytical performance determined on the assay hsa-miR-223-3p was following:

- calibration range 12,5 - 0,39 amol/ul (Fig.2)
- limit of detection 0,13 amol/ul
- intra-assay (n=8) CV= 8,0 %
- spiking recovery 98,1 %
- dilution linearity 101,6 %
- repeatability CV = 12 % on average (Fig. 3)

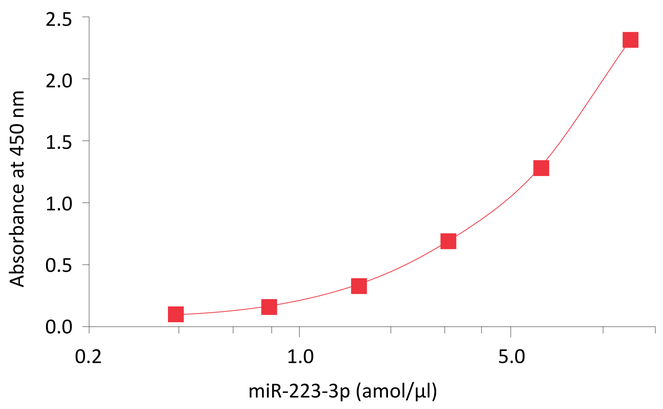


Fig. 2: Typical miREIA standard curve – similar to ELISA principle the absorbance is proportional to the absolute concentration of target miRNA

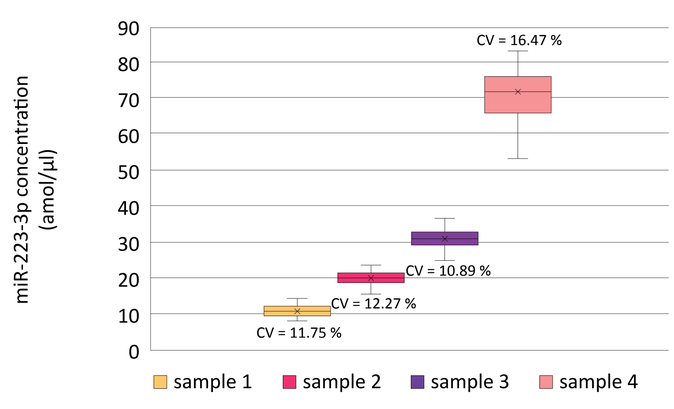
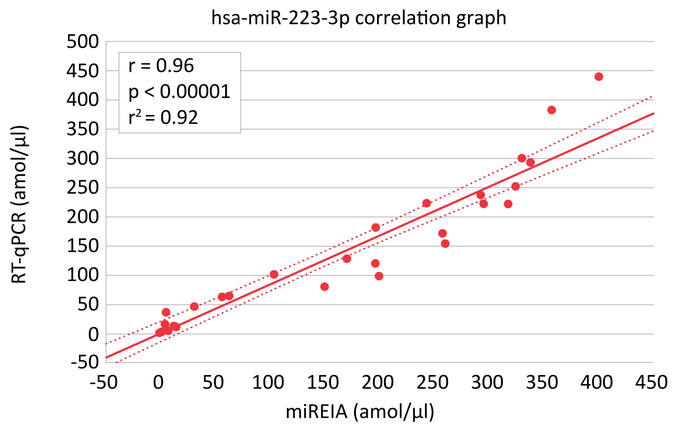


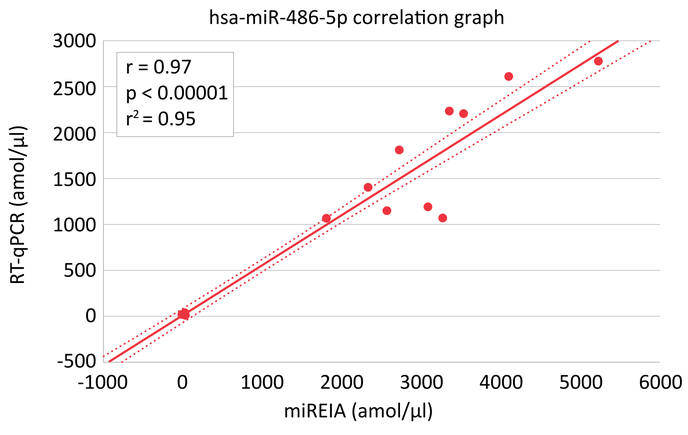
Fig.3: Repeatability – mesaured in 20 consecutive experiments on 4 different concentrations showed low variation what supports miREIA as a good validation tool and potentially applicable method in clinical practice.

CORRELATION

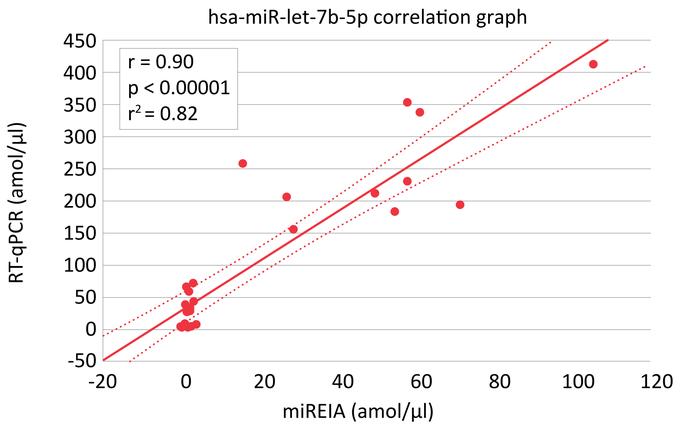
RNA from 38 samples (brain tissue, PBMC, whole blood and plasma) was isolated and analyzed by novel miREIA method and RT-qPCR. We found high correlation between both methods (Graphs 1-3).



Graph 1: correlation of hsa-miR-223-3p



Graph 2: correlation of hsa-miR-486-5p



Graph 3: correlation of hsa-miR-let-7b-5p

CONCLUSION

- miREIA has favourable analytical characteristics.
- Great correlation with RT-qPCR technique measurning 3 stroke related miRNAs.
- Method is well compatible with classic clinical workflow and could help with validation of stroke biomarkers.

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Supported by the European Regional Development Fund
- project „INBIO" (No. CZ.02.1.01/0.0/0.0/16_026/0008451).

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