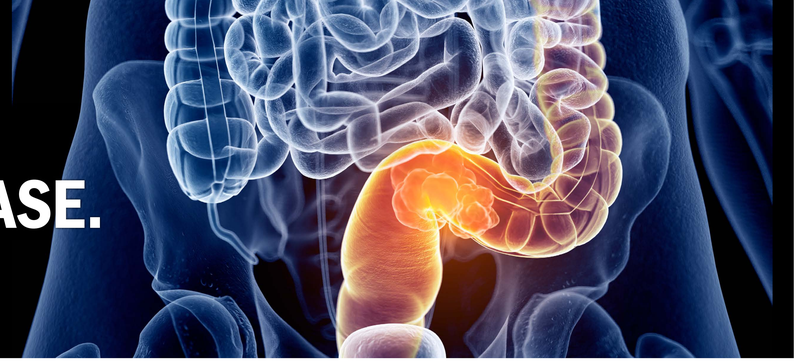
Abstract Number: LB-388

NEW CLINICAL DIAGNOSTIC APPROACH FOR miRNA QUANTIFICATION USING THE *CHLORELLA VIRUS* DNA LIGASE.



Hynštová I.[1,2], Mráčková T.[1,3], Adamcová M.[1], Dvořáková B.[1], Šťastná Z.[1], Slabý O.[2], Bartoš M.[1], Růžička, V.[1]

1 Department of molecular diagnostics, BioVendor – Laboratorní medicína a.s., Brno, Czech Republic · 2 Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Faculty of Medicine, Masaryk University, Brno, Czech Republic

3 Neurology Department, International Clinical Research Center, St Anne's Hospital, Brno, Czech Republic

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 have an immense potential to serve as diagnostic, prognostic and prediction biomarkers in the whole field of oncology. Their utilization in liquid biopsy as a non-invasive diagnostic tool is very promising as well [1].

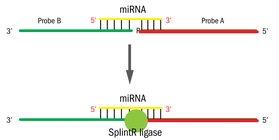
miRNA expression can be measured by many techniques; the three most common being microarrays, next generation sequencing and reverse transcription quantitative PCR (RT-qPCR), which is considered to be a gold standard in miRNA detection [2]. To our knowledge, these methods are not suitable for clinical use – they are low in reproducibility and sensitivity, high technology demanding or very time consuming. New principle-based methods are emerging to overcome these problems, for example immunoassay-based method [3], called miREIA (BioVendor – Laboratorní medicína a.s.).

Another promising technology is utilizing the enzyme Chlorella virus DNA ligase (SplintR® ligase, New England Biolabs) which has recently been found to efficiently ligate two DNA oligonucleotides splinted by RNA [4]. This enzyme has been used for detection of multiple miRNAs by ligation of two partially complementary probes and following qPCR [5]. Our aim was optimized this methodology for potential clinical use.

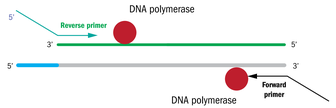
Methods

1 SplintR® ligation PCR

A) Hybridization and ligation

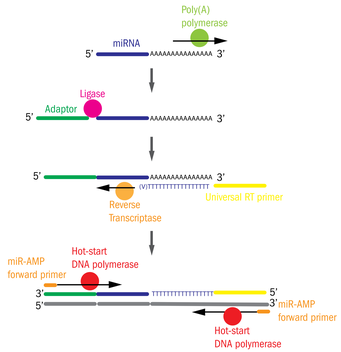


B) Real-time PCR with Syber Green or probe visualization

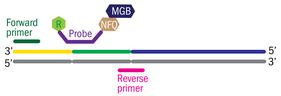


2 TaqMan ADVANCED (Thermofisher Scientific)

A) Reverse transcription

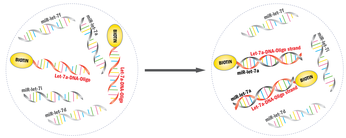


B) Real-time PCR

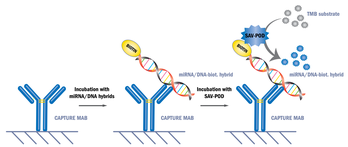


3 miREIA (BioVendor – Laboratorní medicína a.s.)

A) Hybridization

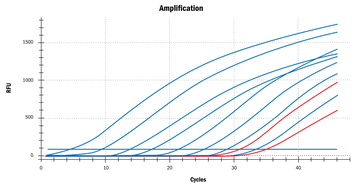


B) Visualization



Results

Figure 1: Amplification plot and standard curve of SplintR® ligation qPCR for detection of miR-142-5p. Synthetic miRNA 142-5p was serially diluted in water. (Blue – standard, Red – no template controls). Dynamic range was determined to 7 logs and sensitivity to 1 amol/µl in original clinical sample.



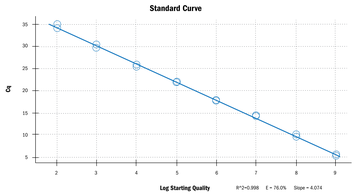


Figure 2: Comparison of Syber Green and Cy5 labeled probe detection mechanisms for miR-142-5p SplintR® ligation PCR. Samples: RNA isolates from whole blood (N =25), peripheral blood mononuclear cells (N = 13) and plasma (N = 11) of healthy donors.

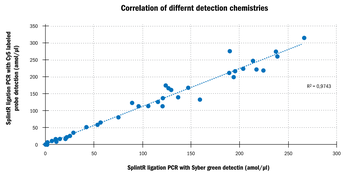


Figure 3: Comparison of miR-142-5p expression measured with SplintR® ligation qPCR and TaqMan ADVANCED method. Samples: RNA isolates from peripheral blood mononuclear cells (N = 13) of healthy donors.

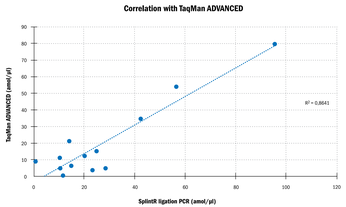


Figure 4: Comparison of miR-142-5p expression measured with SplintR® ligation qPCR and miREIA immunoassay method. Samples: RNA isolates from whole blood (N =25), peripheral blood mononuclear cells (N = 13) and plasma (N = 11) of healthy donors.

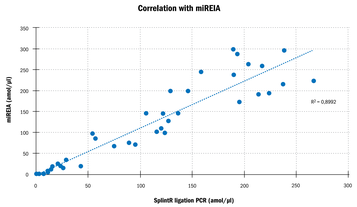


Figure 5: Comparison of parameters of three tested methods for miR-142-5p quantification.

	SplintR® ligation PCR	TaqMan ADVANCED	miREIA
Method principle	Ligation + qPCR	Reverse transcription + qPCR	Hybridization + imunoassay
Dynamic range	7 logs	6 logs	12,5 – 0,39 amol/µl
Sensitivity in original clinical sample	1 amol/µl	5 zmol/µl	0,13 amol/µl
Sample volume	1 µl	2 µl	20 µl
Absolute quantification	Yes	Problematic	Yes
Time to result	2,25 h	4 h	2 h

Conclusion

We have optimised SplintR® ligation PCR for clinical use and developed an assay for detection of miR-142-5p, potential marker of colorectal cancer. Optimized SplintR® ligation PCR showed excellent correlation with TaqMan qPCR and miREIA.

Acknowledgement

This work was funded by the Ministry of Industry and Trade of Czech Republic, project No. CZ.01.1.02/0.0/0.0/16_084/0008832.

References:

[1] M. Redova, J. Sana, and O. Slaby, Circulating miRNAs as new blood-based biomarkers for solid cancers, Futur. Oncol., vol. 9, no. 3, pp. 387–402, 2013.
[2] E. A. Hunt, D. Broyles, T. Head, and S. K. Deo, MicroRNA Detection: Current Technology and Research Strategies, Annu. Rev. Anal. Chem., vol. 8, no. 1, pp. 217–237, 2015.
[3] A. Kappel et al., MicroRNA in vitro diagnostics using immunoassay analyzers., Clin. Chem., vol. 61, no. 4, pp. 600–7, Apr. 2015.
[4] G. J. S. Lohman, Y. Zhang, A. M. Zhelkovsky, E. J. Cantor, and T. C. Evans, Efficient DNA ligation in DNA–RNA hybrid helices by Chlorella virus DNA ligase, Nucleic Acids Res., vol. 42, no. 3, pp. 1831–1844, Feb. 2014.
[5] J. Jin, S. Vaud, A. M. Zhelkovsky, J. Posfai, and L. A. McReynolds, Sensitive and specific miRNA detection method using SplintR Ligase, Nucleic Acids Res., vol. 44, no. 13, p. e116, 2016.

BioVendor
Research and Diagnostic Products