Thoracic Aortic Aneurysm Detection

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Abstract

MicroRNAs (miRNAs), small non-coding molecules, regulate translation and are in many pathological processes. They have emerged as promising biomarkers for diagnosis of conditions such as aortic aneurysm disease. Quantifying miRNAs in plasma is uniquely challenging due to the lack of standardized reproducible protocols. I present a comprehensive protocol for quantifying plasma miRNAs using droplet digital PCR: blood collection, plasma processing, cryo-storage, miRNA isolation, reverse transcription, droplet generation, PCR amplification, fluorescence reading, and data analysis. To facilitate standardization in data reporting, I demonstrate expected results and provide a table of anticipated TAA-related miRNA levels in healthy plasma. This protocol facilitates standardization making it being flexible for lots of diverse and rare circulating miRNAs. Thus, this makes it profitable for TAA diagnoses, where TAA is defined as a 50% or greater dilation of the aortic wall.

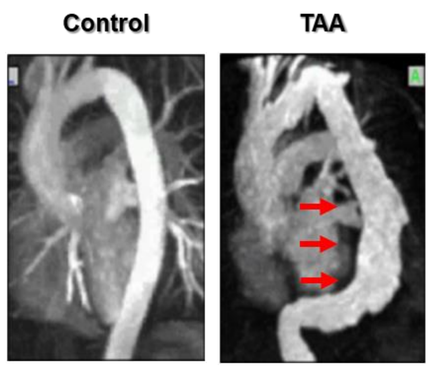


Figure 1. Comparison of normal aortic wall tension vs. Thoracic Aortic Aneurysm.

Project Goal

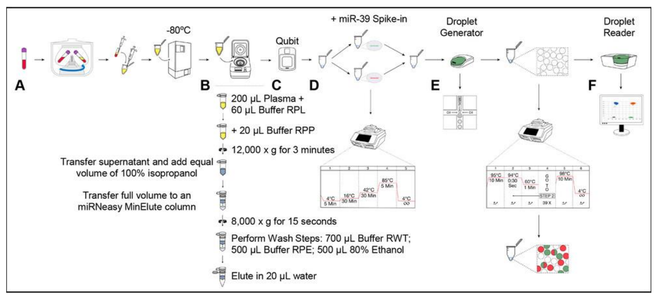


Figure 2. Schematic diagram of the microRNA quantification workflow.

- ❑ Establish a standardized protocol for quantifying plasma microRNAs

RNA Isolation (B)

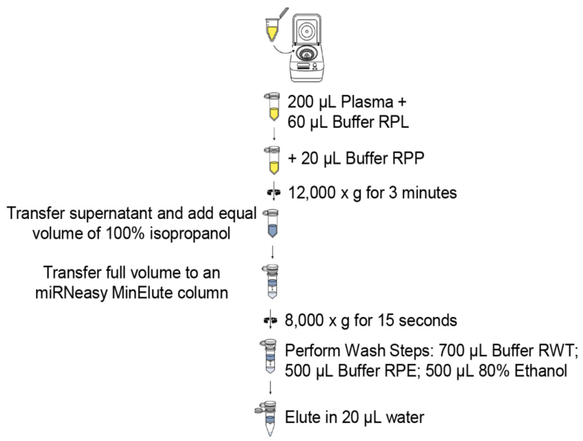
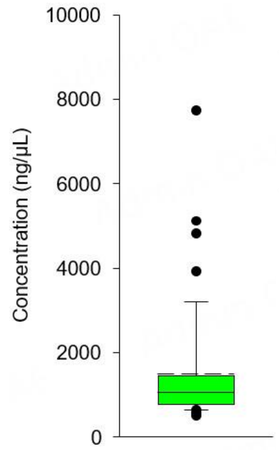


Figure 3. Small RNA Isolation from Plasma. microRNA is isolated according to the manufacturer's instructions of the Qiagen miRNeasy Serum/Plasma Advanced Kit.

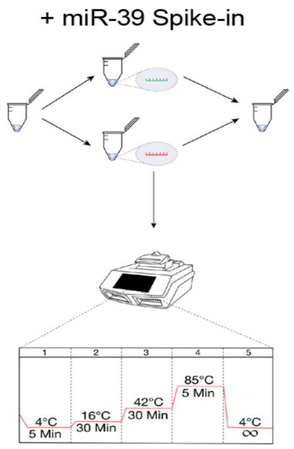
mRNA Quantification (C)



- ❖ Significantly lower or higher samples might indicate issues and should be excluded.
- ❖ Based on measured concentration, isolated miRNA is diluted to a standardized concentration for downstream analysis.

Figure 4. Expected range of microRNA concentration in healthy individuals following isolation quantified by the Qubit microRNA assay.

miRNA to cDNA Generation (D)



- ❖ TaqMan microRNA Reverse Transcription Kit.
- ❖ Two separate cDNA reactions are performed for each target miRNA:
- ❖ One reaction amplifies the target miRNA.
- ❖ Another reaction amplifies the miR-39 spike-in.
- ❖ Reactions performed separately to avoid cross-reactivity.

Figure 5. Synthetic miR-39 (control) spike-in addition and microRNA specific cDNA generation.

ddPCR Amplification (E)

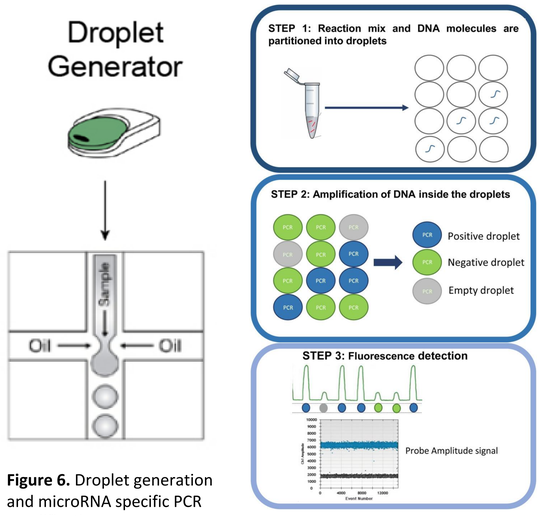
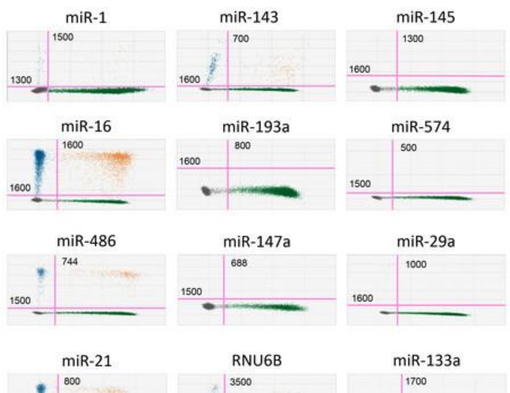


Figure 6. Droplet generation and microRNA specific PCR amplification

Figure 7. Schematic of ddPCR.

ddPCR Analysis (F)



Ratio Scale Normalization

$$\frac{ax_1, bx_1, cx_1, \ldots nx_1}{\sqrt{\sum \left(ax_1^2 + bx_1^2 + cx_1^2 + \ldots nx_1^2\right)}}$$

Equation 1. Congruence transformation of a matrix of values to normalize miR-39 values.

- ❖ Corrects variations in miR-39 spike-in amounts.
- ❖ Adjusts target miRNA measurements for consistent comparisons.

Applications

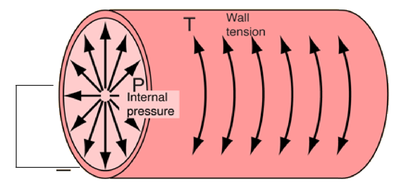
Table 1. Referent plasma microRNA levels in healthy people

Abbreviation	Assay ID	Accession number	Sample size *n* (female, male)	Age (Avg. ± SEM)	Mean normalized concentration (Avg. ± SEM)
miR-1	002222	MIMAT0000416	*n* = 26 (13, 13)	37.96 ± 3.18	5.2817 ± 1.4331
miR-133a	002246	MIMAT0000427	*n* = 20 (11, 8)	40.05 ± 3.54	7.7218 ± 3.2159
miR-143	002249	MIMAT0000435	*n* = 26 (13, 13)	39.38 ± 3.37	56.1333 ± 11.0116
miR-145	002149	MIMAT0004601	*n* = 26 (13, 13)	37.96 ± 3.18	2.9591 ± 1.0029
miR-16	000391	MIMAT0000069	*n* = 26 (13, 13)	39.46 ± 3.19	1,786.6109 ± 547.6928
miR-193a	002250	MIMAT0000459	*n* = 26 (13, 13)	37.96 ± 3.18	3.5471 ± 1.2057
miR-21	000397	MIMAT0000076	*n* = 26 (13, 13)	39.03 ± 3.26	332.7083 ± 83.2669
miR-29a	002112	MIMAT0000086	*n* = 26 (13, 13)	39.53 ± 3.34	37.3906 ± 12.4067
miR-30b	000602	MIMAT0000420	*n* = 26 (12, 14)	41.00 ± 2.67	4,895.2872 ± 1,342.2487
miR-574	002349	MIMAT003239	*n* = 26 (12, 14)	40.57 ± 3.24	7.1525 ± 1.5354
miR-147a	000469	MIMAT0000251	*n* = 25 (12, 13)	41.56 ± 3.21	0.2082 ± 0.0646
miR-486	001278	MIMAT0002177	*n* = 26 12, 14)	40.57 ± 3.24	785.2370 ± 292.3945
RNU6B	001973	NR_004394	*n* = 26 (11, 15)	39.43 ± 3.09	53.0385 ± 26.6712

Researchers can account for potential sex-based variations in miRNA expression. The reference ranges can be further refined based on age groups if needed. Accurate miRNA quantification is crucial for:

- ➢ *Identifying* potential miRNA biomarkers for TAA diagnosis.
- ➢ *Comparing* miRNA expression in healthy vs. TAA individuals.
- ➢ *Evaluating* the role of miRNAs in TAA development/progression.

Aortic Wall Tension = (Pressure) X (Radius)



Acknowledgements

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References

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