RNA and miRNA stability in PAXgene-fixed paraffin-embedded tissue blocks after seven years of storage

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Introduction

Tissue blocks fixed using the PAXgene Tissue fixation system then embedded in paraffin (PFPE) are amenable to immunohistochemistry and offer improved preservation of nucleic acids compared to formalin (Mathieson et al., 2016). We now evaluate the stability of RNA and miRNA in PFPE blocks after seven years of storage at room temperature.

Methods

RNA and miRNA were extracted in 2009 from PFPE blocks of clinical biospecimens plus two cell line controls, using the PAXgene Tissue RNA and miRNA kits. The PFPE blocks were stored at room temperature in the dark and the purified RNA and miRNA were stored at -80°C, after RIN assessment using an Agilent Bioanalyzer. Seven years later, RNA and miRNA were again extracted from the same blocks using the same methods. RNA integrity in the 2009 and 2016 extractions were compared using: RIN (n = 17); PERM (Chung et al., 2016) with RNA quantity normalised in the Bioanalyzer chips, but then additionally normalised post-run to the Bioanalyzer-returned RNA concentrations (n = 8); RT-PCR for 65-942 bp amplicons of the HMBS gene (n = 18) and qRT-PCR for β-actin, GAPDH and HMBS (n = 8). miRNA was evaluated using qRT-PCR for RNU24, miR-16 and miR-221 (n = 10). The 2009 and 2016 extractions from each block were always assayed together to avoid batch effect. Data were compared using the paired t-test or Wilcoxon Signed Rank Test following a Shapiro-Wilk normality test (Sigma Plot software, v 12.5).

Results

The RNA extracted in 2009 then stored at -80°C had not degraded during the intervening seven years: RINs were the same in 2009 (beginning of storage) and in 2016 (end of storage). However, RNA extracted from the PFPE blocks in 2016 was poorer than it was when extracted from the same blocks in 2009.

Median RINs had reduced from 3.6 to 2.0, $p < 0.001$ (Figure 1)

Normalised PERM had reduced from 0.0185 to 0.0146, $p = 0.008$ (Figure 2)

In RT-PCR, the percentage of PFPE blocks from which amplicons ≥ 584 bp were generated reduced from 78% to 5% (Figure 3)

For qRT-PCR from mRNA, for each of the three genes tested, Cts were higher (denoting more degraded RNA) in the 2016 extractions compared to 2009 extractions (Figure 4): mean ΔCt in 2016 extractions compared to 2009 was 1.2 for actin ($p = 0.04$), 2.5 for GAPDH ($p < 0.001$) and 2.4 for HMBS ($p = 0.002$).

For qRT-PCR from miRNA, Cts were the same in the 2016 extractions compared to the 2009 extractions for each of the miRNAs tested, demonstrating that no degradation had occurred during the intervening seven years of storage (Figure 5).

Conclusions

RNA in PFPE degrades at room temperature storage over seven years in the dark but miRNA appears stable.

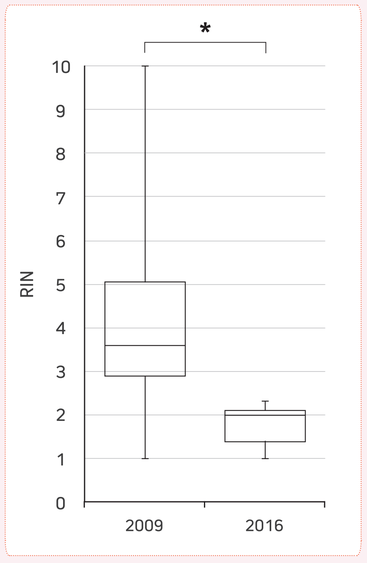

*Figure 1: Mean RNA Integrity Number (RIN) from RNA extracted from the same PFPE blocks in 2009 and in 2016. The box represents the first and third quartiles, intersected by the median and the whiskers are the range. * denotes statistical significance.*

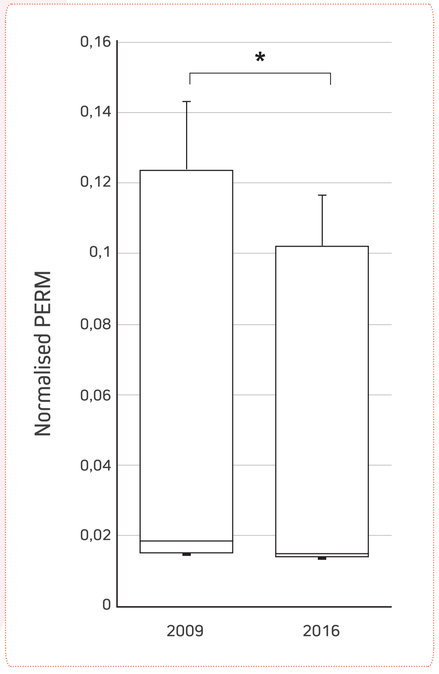

Figure 2: PERM additionally normalised to Bioanalyzer-returned RNA concentrations from RNA extracted from the same PFPE blocks in 2009 and in 2016. The box represents the first and third quartiles, intersected by the median and the whiskers are the range. denotes statistical significance.*

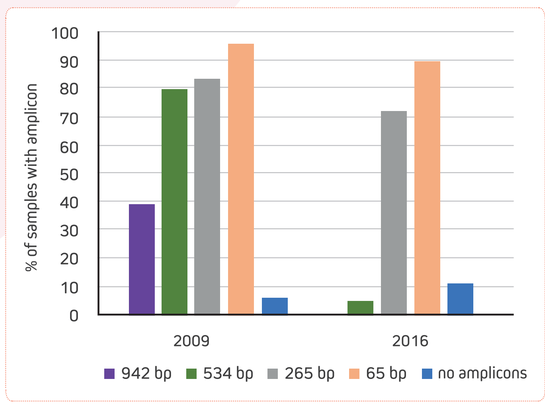

Figure 3: Percentage of samples that generated different RT-PCR amplicon lengths (65, 256, 534 and 942 bp) of the HMBS gene. Samples were RNA extractions from the same PFPE blocks in 2009 and in 2016.

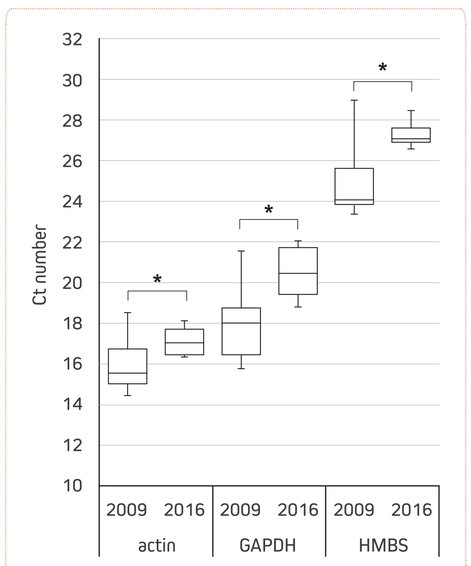

*Figure 4: qRT-PCR of actin, GAPDH and HMBS using RNA extracted from the same PFPE blocks in 2009 and in 2016. For each gene, a higher Ct number denotes more degraded RNA. The box represents the first and third quartiles, intersected by the median and the whiskers are the range. * denotes statistical significance.*

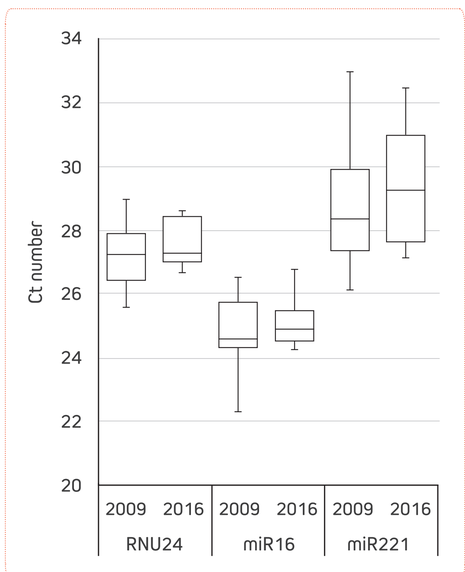

Figure 5: qRT-PCR of RNU24, miR16 and miR221 miRNAs extracted from the same PFPE blocks in 2009 and in 2016. For each of the genes, there were no statistically significant differences in Ct numbers between the two extractions carried out seven years apart. The box represents the first and third quartiles, intersected by the median and the whiskers are the range.

References

Mathieson *et al.*, (2016). Am J Clin Path. **146**(1): 25-40.

Chung *et al.*, (2016). BioTechniques **60**: 239-244.