Preparation of PCR-grade RNA from myocardial biopsy tissue of cardiac surgery patients

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Intact RNA is a key element for the generation of meaningful and reproducible gene expression analysis from real-time PCR. This is especially important for experiments that involve human tissues which are often available in limited supply. The aims of this study are to determine the optimal RNA extraction process for human atrial tissues and to investigate the effects of prolonged tissue storage on real-time PCR gene amplification. Human atrial appendages from patients undergoing coronary artery bypass surgery were pulverized on liquid nitrogen using mortar and pestle and divided into 2 sets of 100 mg aliquots to allow parallel comparison of the RNA preparation obtained using 1) commercial silica-gel membrane based RNA extraction kits (RNeasy Fibrous Tissue Midi kit, Qiagen), which contains an on-column proteinase-k and DNase treatment as part of the protocol, and 2) the 'sequential extraction protocol' where RNA were isolated using a guanidium thiocyanate based cell lysis buffer (TRIzol Reagent, Invitrogen) followed by DNase treatment (Deoxyribonuclease I - amplification grade, Invitrogen) and clean-up (MinElute Cleanup kit, Qiagen). Comparison of the RNA preparations extracted using the two methods have revealed that the sequentially extracted preparations exhibited higher RNA integrity (28S/18S ratio = 2.91 ± 0.31 *vs.* 1.44 ± 0.10, for sequential and kit preparations respectively, $p < 0.001$) and increased RNA purity (RNA Integrity Number, RIN = 9.25 ± 0.19 *vs.* 7.60 ± 0.46, for sequential and kit preparations respectively, $p = 0.009$). Protein contamination was found to be low in both groups (A260/280 ratio = 1.93 ± 0.2 and 1.75 ± 0.1, $p = 0.409$). Investigation of how these RNA measurements are translated to efficient gene amplification were undertaken by performing real-time PCR to assess gene amplification of 18S and GAPDH (two genes that are of rRNA and mRNA origin) in the samples that were extracted using the sequential and kit methods. Despite the variation in RNA integrity and purity, all RNA preparations were found to amplify 18S and GAPDH with similar efficiencies regardless of RNA extraction method (18S amplification efficiency: 1.41 ± 0.04 and 1.47 ± 0.05 for sequential and kit extraction respectively, $p = 0.391$; GAPDH amplification efficiency: 1.86 ± 0.01 and 1.86 ± 0.02 for sequential and kit extraction respectively, $p = 0.577$). All PCR reactions were carried out under pre-established optimized PCR conditions.

The second aim of this study was to investigate the effects of prolonged tissue storage on RNA integrity and real-time PCR gene amplification. RNA was extracted from human atrial appendages, stored at -80ºC for 1 and 5 years designated as 'recent' and 'archived' samples respectively. Similar RNA measurements and gene amplification measurements as described above were assessed. RNA recovery was not different between the archived and recent samples (71.00 ± 10.15 ng/µl *vs.* 74.80 ± 12.95 ng/µl, $p = 0.846$). No differences were found in the RNA integrity measurements including the RIN (8.53 ± 0.07 and 7.04 ± 0.43, for archived and recent samples, $p = 0.124$) and 28S/18S ratio (1.53 ± 0.13 and 1.38 ± 0.14, for archived and recent samples, $p = 0.500$), suggesting similar sample purity and integrity between these sample groups. Real-time PCR was used to assess gene amplification of 18S and GAPDH in the recent and archived samples. All samples were found to exhibit similar amplification efficiency regardless of tissue storage period (18S amplification efficiency: 1.44 ± 0.1 and 1.48 ± 0.05 for archived and recent samples, $p = 0.740$; GAPDH amplification efficiency: 1.85 ± 0.2 and 1.86 ± 0.1 for archived and recent samples, $p = 0.450$).

The present study has established a stringent sample-handling protocol to optimize human gene expression outcomes in human myocardial specimens. Samples extracted using the conventional guanidium thiocyanate based lysis buffer followed by DNase treatment and clean-up have been found to be a better extraction compared to the commercially available silica-gel-membrane based extraction columns. These findings suggest that if simultaneous RNA and protein extraction by TRIzol reagent is desired, extraction of RNA by the sequential method could achieve outcomes that are even better than those achieved by the commercial silica-gel-membrane based RNA extraction kits. In addition, we have found that human atrial tissue samples that have been stored in -80ºC for up to 5 years are suitable for PCR gene expression study.