An optimized RNA extraction protocol for stored human myocardial tissue biopsies

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Human cardiac tissue samples are valuable and availability is limited. Tissue acquisition for batch assay can be protracted and long periods (months to years) can elapse between sample collection and experimental analyses. This is a particular concern for gene expression studies where extended tissue storage may result in RNA degradation, making the tissue unsuitable for PCR analysis. RNA degradation may also occur due to sample exposure to RNases during the RNA extraction process. In order to ensure that real-time PCR results generated from these valuable human samples are reflective of biological difference and not a result of sub-optimal sample preparation method, we have investigated the effects of extended tissue storage and RNA preparation methods on RNA quality and downstream real-time PCR gene amplification. To examine the effects of extended tissue storage on sample quality, right atrial appendages (free from right heart disease) collected from patients undergoing coronary artery bypass surgery and stored at -80ºC for 5 years ('archived') and for up to 1 year ('recent') were compared. RNA extraction was performed using the Qiagen RNeasy® Fibrous Tissue Midi kit. RNA quality was assessed based on the 28S:18S ratio, and the RNA Integrity Number (RIN) - a score ranging from 1 (most degraded) to 10 (most intact). The effects of RNA quality on downstream PCR amplification (real-time PCR) of rRNA and mRNA genes were investigated. RNA recovery was not different between the archived and recent samples (6.70 ± 1.87 *vs* 6.93 ± 1.99 mg total RNA, $p > 0.05$). No differences were found in the RNA integrity measurements including the 28S:18S ratio (2.43 ± 0.17 *vs* 2.50 ± 0.17, $p > 0.05$), and RIN (9.33 ± 0.42 *vs* 9.22 ± 0.25, $p > 0.05$), suggesting that the archived samples were similarly intact compared to the recent samples. This was supported by real-time PCR data where the archived and recent samples were found to exhibit similar amplification of 18S and GAPDH (18S amplification efficiency: 1.48 ± 0.10 *vs* 1.48 ± 0.06, $p > 0.05$; GAPDH amplification efficiency: 1.86 ± 0.01 *vs* 1.86 ± 0.00, $p > 0.05$).

The second aim of this study was to investigate the effects of RNA extraction method on RNA integrity and real-time PCR gene amplification. Frozen human atrial appendages were pulverized using mortar and pestle and divided into two 100 mg portions to allow parallel comparison of the RNA preparation obtained using: 1) a 'multi-step' protocol - where RNA was extracted using phenol/chloroform (TRIzol® Reagent, Invitrogen), followed by DNase treatment (Deoxyribonuclease I - amplification grade, Invitrogen) and purification (MinElute® Cleanup kit, Qiagen); or 2) a 'single-step' protocol – where RNA was extracted using silica membrane based RNA extraction kits (RNeasy® Fibrous Tissue Midi kit, Qiagen), involving on-column DNase treatment and sample purification as part of the protocol. Comparison of RNA preparations extracted using the two methods revealed that the single-step preparations exhibited higher RNA integrity (28S:18S ratio = 1.38 ± 0.12 *vs* 2.50 ± 0.17, $p < 0.05$; RIN = 7.06 ± 0.70 *vs* 9.22 ± 0.25, $p < 0.05$). Consistent with the differences in RNA quality, multi-step samples were found to exhibit compromised GAPDH (mRNA) amplification, while 18S was unchanged. In summary, we have demonstrated that human atrial tissue samples that have been stored at -80ºC for up to 5 years are suitable for PCR gene expression study. In addition, we have established that the single-step RNA extraction method (using silica membrane based RNA extraction kits) is more suitable for human cardiac tissues. These findings suggest that the choice of RNA extraction method is critical and can markedly affect RNA quality and compromise downstream real-time PCR outcomes.