RNA integrity of clinical samples from patients with American cutaneous leishmaniasis and disseminated toxoplasmosis

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Samples of human tissue formalin-fixed and paraffin-embedded (FFPE) are an important source for DNA and RNA extraction. Thus, the development of protocols for RNA extraction from paraffin blocks facilitates studies of gene expression in archived clinical samples with known clinical outcome. The objective this study was to verify by different proceedings whether RNA extracted from FFPE samples present good quality for use in molecular experiments. The analyses were done in 18 skin biopsy samples (FFPE) from patients with American cutaneous leishmaniasis, confirmed by positive immunohistochemistry and PCR; and 22 lung and brain samples (FFPE) from patients who died with disseminated toxoplasmosis. RNA molecules were extracted from FFPE samples using RNeasy FFPE isolation kit (Qiagen), according to the manufacturer's instructions. Next, molecules were treated with DNase. The quality of RNA molecules was determined by: i. quantification by fluorimetry (Quantus) and spectrophotometry (NanoDrop); ii. integrity was determined in an Agilent 2100 Bioanalyzer, whose values were expressed in RNA Integrity Number (RIN); and iii. quality of RNA extraction was evaluated by real-time PCR (qPCR) using a molecular marker that amplified a product from human GAPDH gene which is expressed in most tissues at relative constant level. Before qPCR procedure, RNA molecules were submitted to cDNA synthesis.Among the 40 RNA samples the values were: RNA concentration determined by NanoDrop ranged from 58.9-496.9 ng/µL in lung; 16.6-488.4 ng/µL in brain; and 25.1-318.6 ng/µL in the skin samples. The values determined by Quantus were 33.1-115 ng/µL in lung; 21-109ng/µL in brain and 25.8-197 ng/µL in skin samples. RIN ranged from 2.5-3.1 in lung, 2.1-5.1 in brain, and 2.0-4.1 in skin samples. The mean RIN values obtained was 3.57. All samples had positive qPCR for endogenous gene (GAPDH) with Threshold Cycle (C_T) values ranged from 19.99-36.03. The major limitation of FFPE samples is RNA degradation that normally occurs prior to and during formalin fixation. Usually, these RNA molecules are fragmented in units smaller than 300 pairs and with low RIN. However, when experiments are made by qPCR, products can be amplified properly. In the literature, cut-off values of RIN $\geq$5 are found for different tissues and cell cultures. For human brain tissue, they calculated the RIN threshold $\geq$ 3.95. The mean RIN obtained in a study of FFPE colon tissue was 2.57 same result obtained by our group. Therefore, our study demonstrated that RNA extracted from FFPE samples using the QIAGEN FFPE kit is efficient in quantity and quality appropriate for a molecular biology analysis of gene expression.

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