Abstract

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Title of diploma thesis: Influence of Carrier Molecule Addition on Plasma Free Circulating Nucleic Acid Extraction

Outline: The aim of this thesis was to compare the yields of cell-free DNA (cfDNA) extracted from blood plasma specimens with the addition of commercially available carrier molecules. According to the real-time polymerase chain reaction (qPCR) results, we chose the most optimal carrier molecules for routine isolation of cfDNA from blood plasma samples.

Methods: cfDNA was isolated from aliquots of a pooled blood plasma by spin column extraction method NucleoSpin Plasma XS (Macherey-Nagel). We used 7 different types of carrier molecules as RNA carrier (Qiagen), Glycogen (Invitrogen), Poly (A) Polyadenylic acid (Roche), Linear Acrylamide (Invitrogen), Yeast transfer RNA (Ambion), Salmon sperm DNA (Invitrogen), Herring sperm DNA (Promega). After the extraction, plasmatic DNA with the addition of carrier molecules was quantified by spectrophotometry, fluorimetry and qPCR.

Results: The best results were achieved with the addition of polyadenylic acid with final concentration of 1.55 μg/ml. The addition of carrier RNA and polyadenylic acid with final concentration of 1.55 μg/ml and 0.1 μg/ml did not show any significant effect on the yield of cfDNA. Glycogen, linear acrylamide, yeast tRNA, salmon sperm DNA and herring sperm DNA had a significant negative effect on the yield of cfDNA.

Conclusion: Due to a positive effect of the addition of polyadenylic acid (with final concentration of 1.55 μg/ml) to the amount of eluted cfDNA, this carrier was evaluated as the most suitable carrier for rutine isolation of cfDNA from blood plasma samples by NucleoSpin Plasma XS Kit. The other carrier molecules did not improve the yield of cfDNA, and therefore they are not appropriate for isolation of cfDNA by spin column extraction methods.