

Verification of DNA isolation methods for NGS-HERC analysis

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Abstract

Background-aim: Next generation sequencing (NGS) in oncology starts with high quality starting sample material to obtain reliable NGS results and afterwards accurate data analysis. Here we present short verification of manufacturer data of three different methods of DNA isolation from the same sample in which yield of DNA correlates with lymphocyte number.

Methods: Whole blood samples were collected in EDTA tubes from patients referred for NGS hereditary cancer panel (HERC) testing on Illumina MiSeq platform. We provided numbers on 30 patients. Lymphocyte number was determined on Sysmex XN-1000 analyzer. DNA was isolated from 200 μ L whole blood or buffy coat using Qiagen kit QiaAmp DNA Blood Mini Kit for DNA isolation and also from whole blood on QIAcube. Concentration was measured on NanoDrop™ Lite Spectrophotometer and Qubit4.

Results: According to the obtained results, we established the protocol of using optimal DNA isolation method for NGS analysis in oncology patients. We compared DNA concentration of three different starting samples and methods. If lymphocyte count is lower than $1,0 \times 10^9/L$ optimal sample is buffy coat and QiaAmp method. If lymphocyte count is between $1,0$ and $2,5 \times 10^9/L$ optimal sample is whole blood and QiaAmp method. In cases when lymphocyte count is higher than $2,5 \times 10^9/L$ QIAcube isolation can be proceed to obtain high quality DNA and minimal 30 ng/ μ L DNA concentration sufficient for NGS analysis that follows.

Conclusion: Considering potentially low lymphocyte number in oncology patients we developed optimal protocol for DNA isolation using different starting samples and methods to ensure valid DNA sample for NGS analysis.

Biography

Vanja Raščanec has finished in 2016 Medical biochemistry in Zagreb and currently is on specialization of medical biochemistry and laboratory medicine in KBC Zagreb. For past two year is working in field of molecular biochemistry and medicine, and currently developing NGS protocol for hereditary cancer research to incorporate in laboratory practice.