ApplicationForum

Purification and quantification of circulating cell-free DNA from body fluids with DANAGENE Circulating System applied to Liquid Biopsy

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Introduction

The phenomenon of increased concentrations of circulating cell-free DNA (cfDNA) is considered a hallmark of various pathological conditions like cancer, autoimmune diseases, infectious diseases, stroke , sepsis, trauma and pregnancy.

Quantification of plasma cfDNA has been proposed as a diagnostic tool for cancer. The quantity of cfDNA is generally very low in healthy subjects (less than 5ng/ml of plasma) and increases (8 to 10 times) when considering subjects affected by a neoplastic disease, as well as in some physiological conditions.

Therefore, the development of reproducible, standaridzed methods for the detection and quantification of cfDNA is important for improving the sensitivity, specifity and relevance of this biomarker.

The isolation and quantification of cfDNA from body fluids represents a challenge, due to their small quantity and fragmented nature.

DANAGEN-BIOTED has developed a system for isolation of cfDNA for samples of 1 or 3 ml from body fluids using Midi Spin columns with a special resins that bound cfDNA.

Sample	Ct	Copies on assay	Sample concentration (copies/ul)
1	22.34	6.8E+04	1.4E+04
2	21.18	1.4E+05	2.8E+04
3	20.67	2.0E+05	4.0E+04
4	22.21	7.4E+04	1.5E+04
5	22.43	6.4E+04	1.3E+04
6	20.82	1.8E+05	3.6E+04
7	23.30	3.6E+04	7.2E+03
8	21.33	1.3E+05	2.6E+04
9	26.31	5.0E+03	1.0E+03
10	28.46	1.2E+03	2.4E+02
11	20.78	1.9E+05	3.8E+04
12	19.47	4.5E+05	9.0E+04

Table 1. Quantification of cfDNA from plasma samples

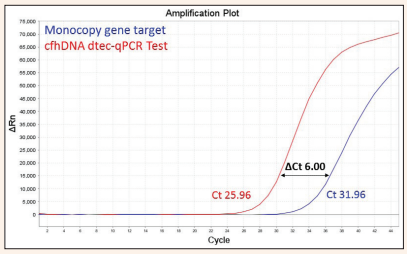

Figure 1. Real-time PCR amplification plot for cfhDNA dtec-qPCR Test (red) targeting a "non-truncated" multi-copy gene and compared to a monocopy target (blue), using a human genomic DNA as a standard. Due to the presence of multiple copies of the selected target, sensibility is increased 2 logs (100 times) for the cfhDNA dtec-qPCR Test. Same increased signal is observed for the purified cell-free DNA samples employed for cell-free DNA quantification.

Materials and Methods

Circulating Cell-Free DNA Isolation

Blood samples were collected from 8 patients (samples 1 to 8) with breast cancer and healthy controls.

2 samples were used for healthy individuals (sample 9 and 10) and 2 samples of healthy individuals were spiked with 150 ng (sample 11) and 300 ng (sample 12) of human genomic DNA. Plasma was carefully separated and stored at -80ºC. The stored plasma was thawed at room temperature and centrifuged at 15.000 x g to remove residual precipitated cellular components.

Circulating cell-free DNA was extracted from 3 ml of plasma following **DANAGENE Circulating DNA Kit** protocol.

Quantification of Circulating Cell-Free DNA

It was quantified the total amount of cf DNA isolated using the **Cell-free human (cfh) DNA dtec-qPCR Test** developed by Genetic PCR Solutions™ (Alicante, Spain). The cfhDNA dtec-qPCR Test was designed to target a conserve sequence region of a gene repeated more than a hundred times in the human genome. qPCR, total volume of 20 ul qPCR mixture was prepared by adding 4 ul of MixStable qPCR.5x (GPSTM, Spain), 10 ul of nuclease free water, 1 ul of the primers/probe reagent (reagents included in the kit), and 5 ul of purified samples, following the instructions of the manufacturer. The real-time PCR thermal protocol used for amplification of the target gene, as recommended by the manufacturer, was: activation step at 95 ºC for 15 minutes, followed by 40 cycles of denaturation at 95 ºC for 15 seconds and annealing/extension at 60 ºC for 60 seconds. Data collection was performed during annealing/extension step by using the FAM channel.

Results

cfDNA were quantified by realtime PCR. The measured threshold cycle (Ct) and copies are listed in Table1. We successfully detected cfDNA in all cancer patients. Our results are consistent with others, and have shown that cfDNA can be detected in subjects with cancer.

Conclusion

The quantitative analysis of plasma DNA may be useful in distinguishing patients with cancer from healthy individuals.

In this paper, has been demonstrated the sensitivity in the quantification of cfDNA from plasma using DANAGENE Circulating DNA kit and Cell free human DNA dtec-qPCR Test.

This method will also be beneficial for isolating cfDNA in other pathological conditions. ctDNA collected without percutaneous tumor biopsy, also known as Liquid Biopsy, can become an innovative tool to analyze the cancer genome with obvious clinical importance for personalized treatment of cancer.