Quality Analysis of Circulating Cell-Free DNA from Indivumed's Plasma Sample Collection

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Cell-free circulating DNA (cfDNA) fragments originate from both normal and abnormal (tumor) cells via various mechanisms such as apoptosis, necrosis, and active secretion, and can be found in various body fluids, including blood plasma, urine, sputum, cerebrospinal fluid, pleural fluid, cyst fluid, and saliva [1]. Molecular analysis of cell-free tumor DNA (ctDNA) from liquid biopsies is gaining importance in various oncology-related clinical applications such as diagnosis, prognosis, therapy selection, and/or monitoring.

To support the research and development of various cfDNA biomarker assessment platforms, Indivumed has implemented blood collection and plasma processing procedures to meet the need for high-quality cfDNA. In this study, we evaluated the quality and quantity of plasma cfDNA isolated from blood collected from various patient's within Indivumed's Clinical Network.

Keywords:
- Liquid biopsy
- Cell-free DNA
- qPCR

Material and Methods

Blood from various colorectal cancer (CRC) patients was collected and processed accordingly:

Blood collection and plasma preparation:

- Blood from patients was collected using the indicated tubes (EDTA or Streck)
- Blood collected in EDTA or Streck tubes was processed according to Standard Operating Procedure by using a single spun or double spun plasma preparation protocol
- Plasma samples were aliquoted and stored at -80°C

Site #	Sample Source	n	Tube Type	Blood Spin Protocol
1	Treatment-naïve	10	K_3- EDTA	Single
2	Treatment-naïve	10	K_3- EDTA	Double
3	Treatment-naïve	9	K_3- EDTA	Double
4	Treatment-naïve	9	K_2- EDTA	Double
5	Treatment-naïve	10	K_2- EDTA	Double
6	Treatment-naïve	10	K_2- EDTA	Double
7	Treatment-naïve	10	K_2- EDTA	Double
8	Adjuvant treated – Longitudinal plasma	10	Streck	Double

cfDNA isolation:

- Frozen plasma samples (1 ml plasma per patient) were thawed at room temperature
- Immediately after thawing, plasma samples were centrifuged for 5 minutes at 4°C and 20,000x g
- Supernatant (plasma) was carefully transferred
- Automated isolation of cfDNA was performed from 1 ml plasma by using the Maxwell® RSC device (Promega)

Methods used for evaluation of concentration, yield, and quality of cfDNA:

- Fluorescence-based capillary electrophoresis for size and quality determination using the Fragment Analyzer (Agilent Technologies)
- qPCR-based measurement for concentration and quality assessment

Results

cfDNA quality:

The size distribution of isolated cfDNA is displayed in an electropherogram view (Figure 2A–2B). Typically, cfDNA shows a fragmentation pattern below 1,000 bp with no DNA peaks above 1,000 bp as indicated in Figure 1A. CfDNA of all double spun plasma samples only showed this pattern without high molecular weight DNA fragments (Figure 2A). Two out of ten single spun plasma samples showed beside the cfDNA fragmentation pattern high molecular weight DNA fragments (Figure 2B). These high molecular weight DNA fragments indicate contamination with genomic DNA (gDNA).

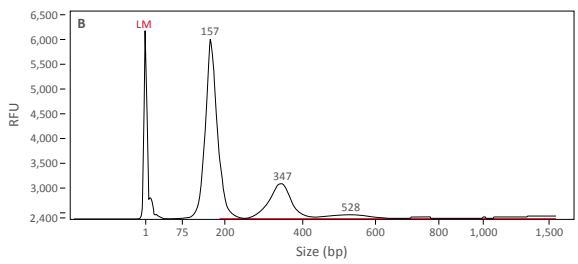

Figure 1: Example from literature regarding cfDNA separation by capillary electrophoresis [2].

To analyze the cfDNA quality and possible gDNA contaminations in more detail, a qPCR-based measurement of three different amplicons with different lengths was performed. The calculation of a ratio (ratio 305/41) serves as an indicator of gDNA contamination. The lower the ratio, the lower the probability of gDNA contamination.

As indicated in Figure 3 all samples collected in EDTA blood tubes and isolated with double spun plasma preparation protocols showed low ratios and these ratios are comparable between samples of all sites.

The lowest ratio and therefore the lowest probability of gDNA contamination was detected in samples isolated from plasma collected in Streck tubes. Nevertheless, differences between cfDNA isolated from EDTA and Streck tubes are only small and not significant. The highest ratio and therefore highest probability of gDNA contamination was detected in samples collected in EDTA blood tubes and isolated with single spun plasma preparation protocol.

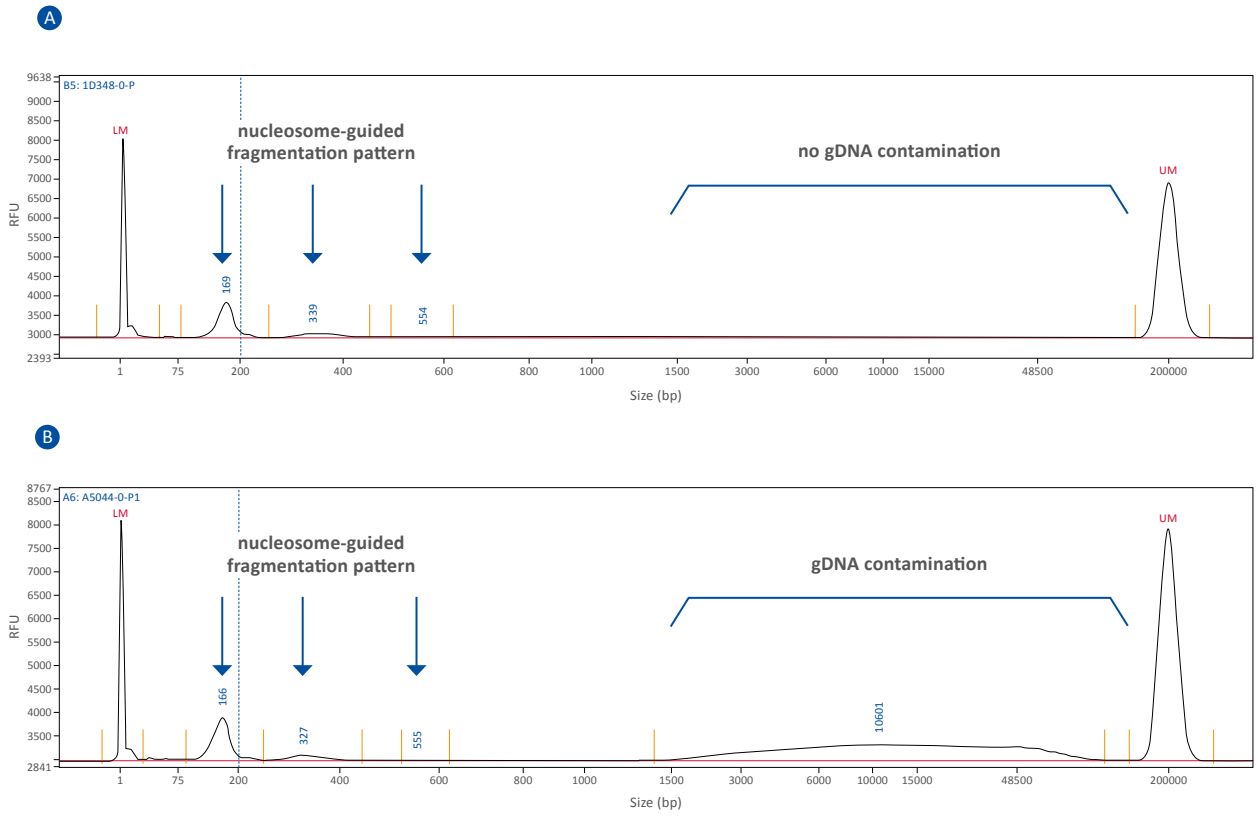

Figure 2: A) Capillary electrophoresis of cfDNA isolated from a double spun plasma sample showing no detectable high molecular weight DNA pattern. B) Capillary electrophoresis of cfDNA isolated from one single spun plasma sample with detectable high molecular weight DNA pattern.

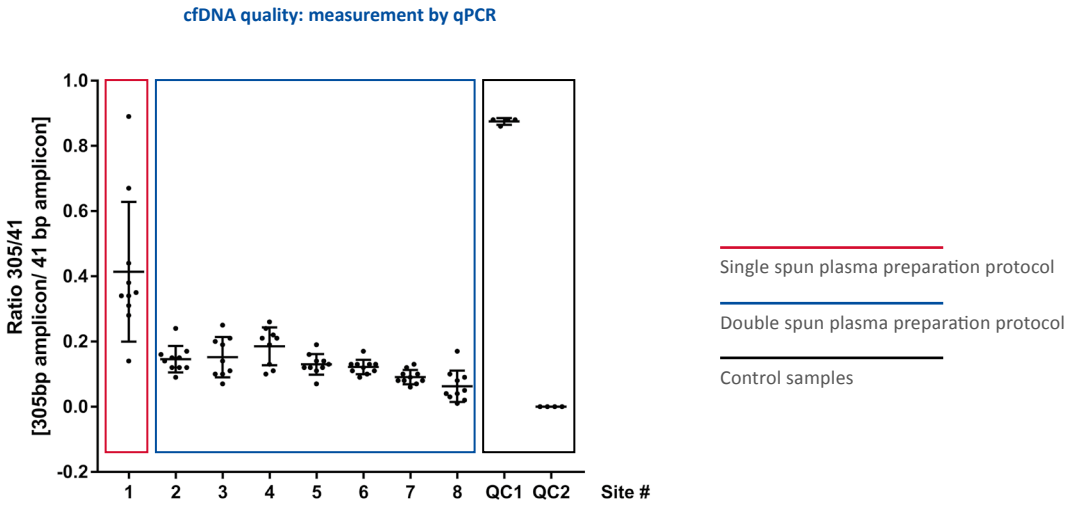

Figure 3: Measurement of cfDNA by determination of ratio 305/41 by qPCR. Ratios are displayed for cfDNA isolated from single spun plasma samples (red box), double spun plasma samples from different sites (blue box) and controls: high-quality control gDNA sample with non-degraded DNA (QC1, Fresh Frozen DNA) and low-quality control sample with fragmented DNA (QC2, FFPE-DNA).

cfDNA concentration/yield:
Most often cfDNA concentrations were determined by using qPCR-based approaches measuring amplicons with different lengths.

The total yield of isolated cfDNA ranges between 3–196 ng of DNA/ml plasma whereas more than 75% of samples showed yields between 5–30 ng DNA/ml plasma (Figure 4A). Furthermore, comparison of cfDNA yields between early (stage I&II) and advanced (stage III&IV) stage CRC patients revealed higher cfDNA yields in patients with advanced stage CRC (mean stage I&II: 13.9 ng/ml plasma versus mean stage III&IV 21.6 ng/ml plasma) (Figure 4B).

Conclusion

Results revealed that isolation of cfDNAs from Indivumed's plasma samples resulted in cfDNAs with concentrations and yields that are comparable and in accordance with results published elsewhere in literature [3]. Furthermore, the quality control revealed low probability of gDNA contamination of cfDNA isolated from double spun plasma samples.

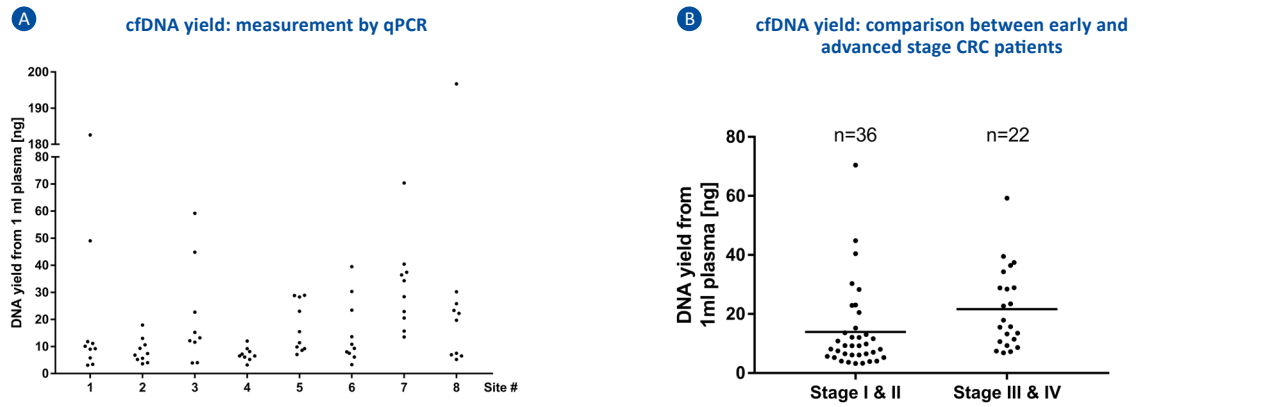

Figure 4: A) Concentration of cfDNA isolated from 1 ml plasma was measured by qPCR and is displayed as yield for all sites. B) Comparison of cfDNA yields between early stage (stage I&II) and advanced stage (III&IV) CRC patients; only results of cfDNA isolated with double spun plasma preparation protocol are displayed.

Literature

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