Monitoring the Impact of Pre-analytical Parameters on cfDNA Quality

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Introduction

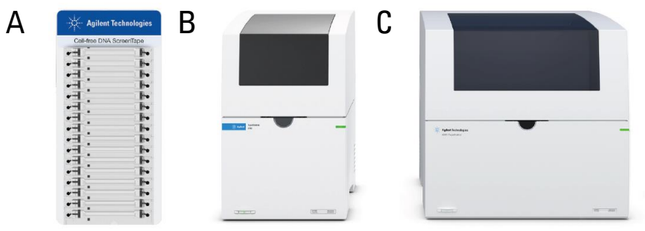
Cell-free DNA (cfDNA) has become an important source for potential new biomarkers. Yet, it represents a challenging sample type due to its low yield and complex fragment size distribution. Controlling and monitoring pre-analytical parameters, including cfDNA extraction, is crucial for experimental success. A new automated electrophoresis assay was used to compare nine different kits tailored for cfDNA extraction.

Experimental

Three independent plasma samples were processed with nine different extraction kits in parallel. CfDNA was extracted from human blood plasma using the following kits: QIAamp Circulating Nucleic Acid kit (Qiagen), QIAamp MinElute ccfDNA kit (Qiagen), GenElute Plasma/Serum Cell-Free Circulating DNA Purification kit (MilliporeSigma), MagMAX Cell-Free DNA Isolation kit (Thermo Fisher Scientific), Quick-cfDNA Serum & Plasma kit (Zymo Research), cfPure Cell Free DNA Extraction kit (BioChain), NucleoSnap DNA Plasma kit (Macherey-Nagel), Plasma/Serum Cell-Free Circulating DNA Purification Kit (Norgen Biotek), and Mag-Bind cfDNA kit (Omega Bio-tek).

Agilent TapeStation systems

The extracted cfDNA samples were analyzed with the Cell-free DNA ScreenTape assay in combination with the 4150 and 4200 TapeStation systems.



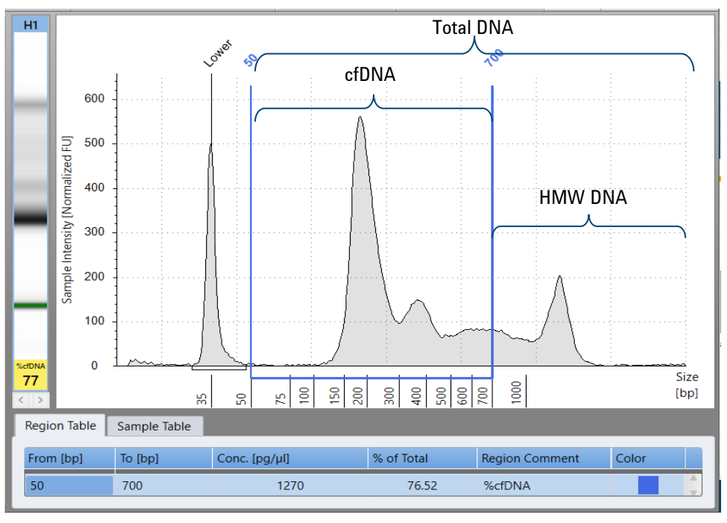
Components of the Agilent TapeStation system.
A) Cell-free DNA ScreenTape device with 16 individual gel lanes. B) 4150 TapeStation instrument for automated electrophoresis of 1-16 samples per run. C) 4200 TapeStation instrument for automated high through-put electrophoresis of up to 96 samples per run.

Conclusions

- The Cell-free DNA ScreenTape assay provides a straightforward way to monitor the impact of pre-analytical parameters on cfDNA concentration and quality.
- In general, each extraction kit afforded either superior yield or high selectivity towards cfDNA.
- The choice for the most suitable cfDNA extraction kit depends on the requirements for downstream analyses as well as the expected cfDNA concentration and occurrence of HMW DNA.
- High yield may be crucial for plasma with low cfDNA content, yet if the plasma is expected to contain considerable amounts of HMW DNA, the selectivity towards cfDNA might be favored.

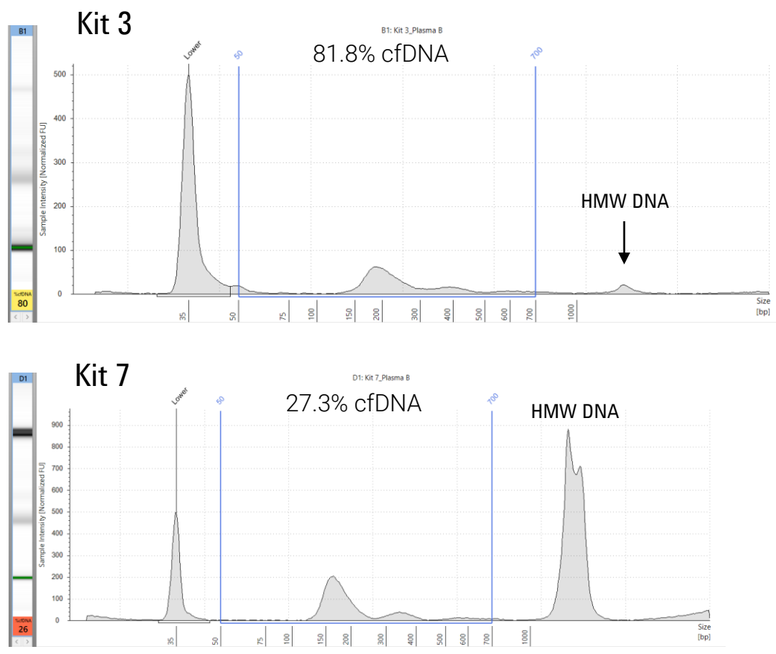
Results and Discussion

The Cell-free DNA ScreenTape assay offers total DNA quantification, separate cfDNA quantification, and a %cfDNA quality score. A pre-set cfDNA region of 50 to 700 bp is assigned to separate cfDNA from high molecular weight (HMW) DNA. Using region analysis, the concentration of cfDNA apart from HMW DNA is automatically evaluated by the software. The %cfDNA quality score is provided as an additional quality parameter, determining the percent cfDNA subcomponents in the total DNA sample. The %cfDNA quality score provides a reference allowing for quick decisions of the cfDNA sample quality.



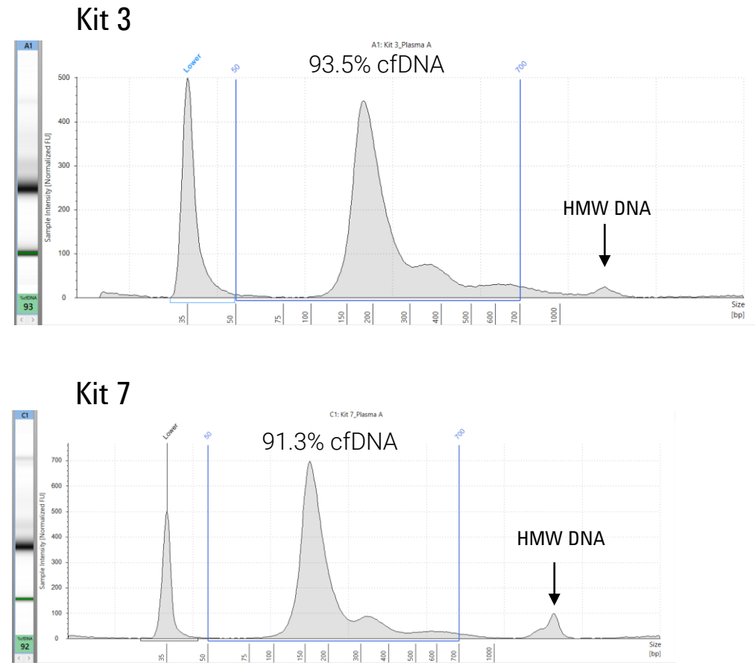
Plasma 2

CfDNA was extracted from plasma 2 with two different kits. Kit 3 was more selective towards cfDNA resulting in a higher %cfDNA quality score compared to Kit 7. A higher %cfDNA correlates with less HMW DNA content, as seen by the smaller HMW DNA peak in Kit 3 compared to Kit 7.



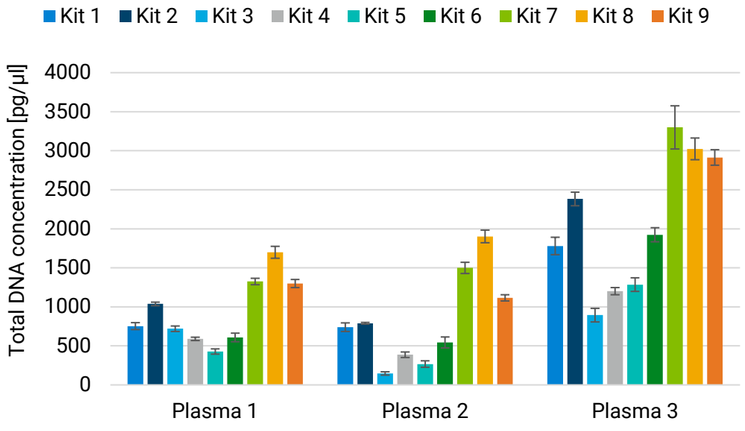
Plasma 1

CfDNA was extracted from plasma 1 with kits 3 and 7. Both kits displayed similar %cfDNA and similar concentrations of HMW DNA. Plasma sample 1 did not require a kit with higher selectivity between cfDNA and HMW DNA because it contained minimal HMW DNA.



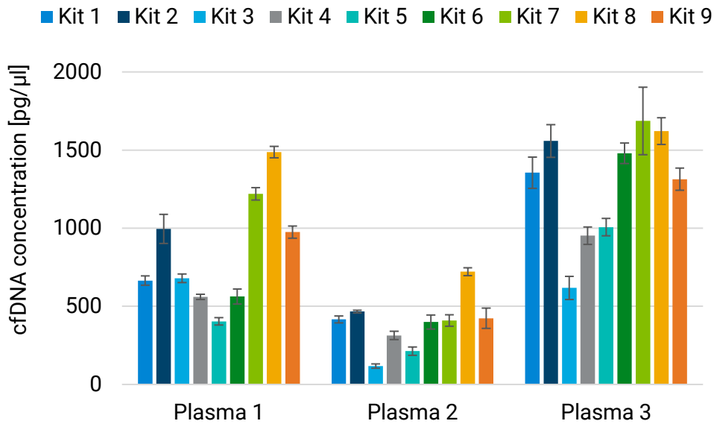
Results and Discussion

A. Total DNA



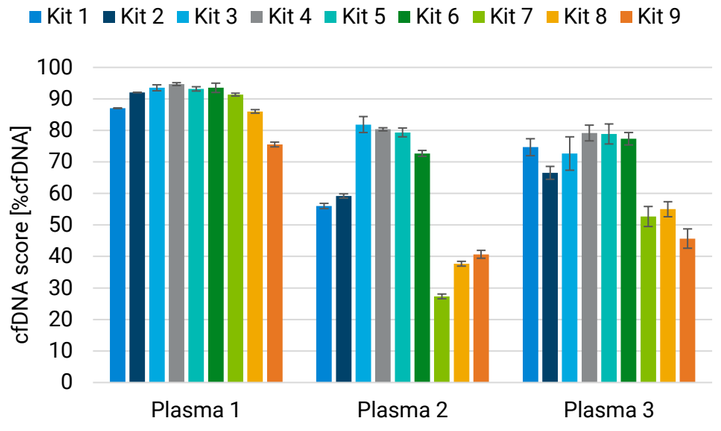
The Cell-free DNA ScreenTape assay in combination with the 4150 and 4200 TapeStation systems was used to analyze DNA extracted from nine different cfDNA extraction kits. (A) Each of the nine kits resulted in varying amounts of total DNA from the same plasma sample. Plasma samples from different origins are known to have varying amounts of total DNA and cfDNA. Comparison of total DNA extracted from three plasma samples demonstrated different amounts of total DNA from the same kit.

B. cfDNA concentration



A pre-set analysis region in the Cell-free DNA ScreenTape assay provides automatic quantification of cfDNA separate from HMW DNA and a %cfDNA quality score. The pre-set region of 50 to 700 bp allows for analysis of only cfDNA. (B) Each of the nine kits resulted in varying amounts of cfDNA from the same plasma sample. Comparison of three plasma samples demonstrated different amounts of cfDNA from the same kit.

C. %cfDNA score



(C) Plasma sample 1 displayed a high %cfDNA with all nine kits indicating very low amounts of HMW DNA in the sample. In contrast, the%cfDNA in plasma sample 2 and 3 varied greatly between the nine extraction kits. This reveals that sample 2 contains larger amounts of HMW DNA and that the extraction kits differ in their selectivity of pulling down cfDNA without HMW DNA.

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