Automated Large-Volume Extraction of Circulating, Cell-free DNA Using the QIAsymphony SP Instrument

Martin Horlitz, Annette Nocon, Lena Albers, Berthold Lechtenberg, Markus Sprenger-Haussels

QIAGEN GmbH, R&D Department, 40724 Hilden, Germany | martin.horlitz@qiagen.com

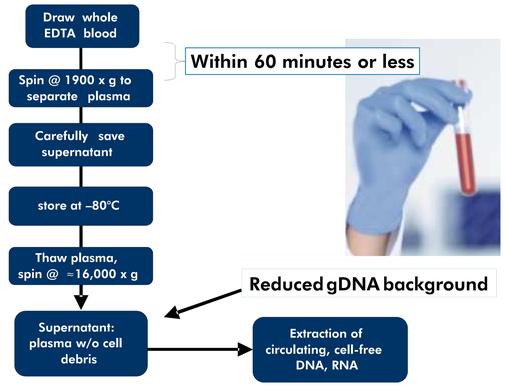
Introduction

Fetal circulating, cell-free DNA (ccfDNA), present in a pregnant woman's blood plasma, has become a crucial analyte for non-invasive prenatal diagnostics (NIPD). Because of its extremely low concentration (less than 20–50 ng/ml plasma) and high degree of fragmentation, the extraction of ccfDNA is technically challenging. Here, the efficiency of a new automated large volume ccfDNA extraction method, and a modification of an existing protocol, was evaluated against a manual reference method.

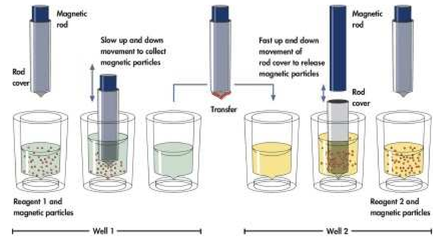
EDTA plasma from healthy individual donors (with donor consent) was used for the development of (a) a new ccfDNA enrichment protocol involving magnetic particles with novel surface chemistry and (b) a modified automated extraction protocol (QIAsymphony DSP Virus/Pathogen Midi Kit), both running on the QIAsymphony SP instrument. Plasma (4–5 ml) was extracted, and ccfDNA eluted in a final volume of 60–150 µl. The QIAamp® Circulating Nucleic Acid Kit (QIAamp CNA Kit) served as reference method to determine the amount of ccfDNA as quantified by qPCR. As internal control, DNA fragments (75, 200, 1000 bp) were added to the samples and recoveries were measured by qPCR.

The applications presented here are for research purposes. Not for use in diagnostic procedures.

Methods: Blood Sample Processing

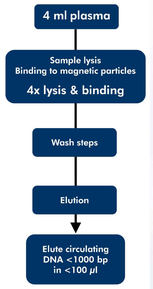


The QIAsymphony Principle



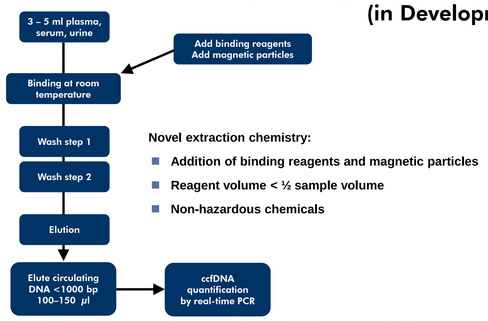
Schematic of the QIAsymphony® SP principle.
The QIAsymphony® SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony® SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

Automated Extraction using Existing QIAsymphony Kit & Modified Protocol



The QIAsymphony DSP Virus/Pathogen Midi Kit allows for a maximum sample volume of 1 ml by default using the „virus cell-free 1000" protocol. This protocol was modified to allow for processing of 4 ml cell-free sample (e.g., plasma) using the same extraction kit. The elution volume (of 60 µl) remained unchanged.

Novel Extraction Chemistry for Large-Volume Samples (in Development)

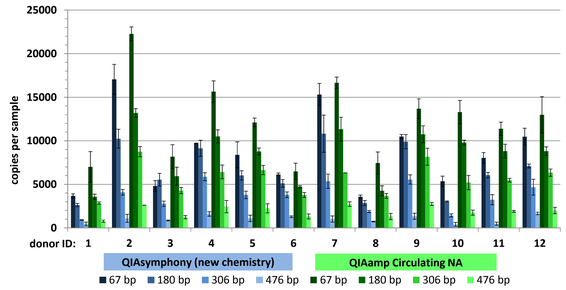


Novel extraction chemistry:

- Addition of binding reagents and magnetic particles
- Reagent volume < ½ sample volume
- Non-hazardous chemicals

- Up to 96 samples are processed in one QIAsymphony® SP run
- Magnetic particles are washed to remove PCR inhibitory factors
- Bound DNA is eluted in Tris buffer
- All steps are performed at room temperature

Results: Novel Extraction Chemistry – ccfDNA Yields



Circulating cell-free DNA was purified from 5 ml plasma from 12 individual donors using the novel enrichment protocol on the QIAsymphony SP instrument (elution in 150 µl). DNA yield was quantified by real-time PCR targeting four amplicons in the APP gene (67 bp, 180 bp, 306 bp, 476 bp)* using the QIAGEN QuantiTect® Multiplex PCR Kit. The QIAamp® Circulating Nucleic Acid Kit (QIAGEN) served as reference method. *as published by P. Pinzani *et al.*, Clinica Chimica Acta 412 (2011) 2141–2145

Extraction of circulating DNA on the QIAsymphony SP with the QIAsymphony DSP Virus/Pathogen Midi Kit

Modified Protocol for 4 ml Plasma/Serum

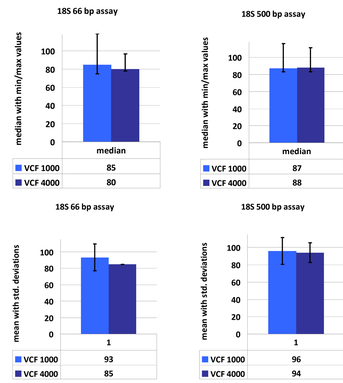
Set-up of experiment: Blood samples from 5 healthy donors, plasma separated from whole blood by double centrifugation — Methods compared:

- QIAamp Circulating Nucleic Acid Kit as manual reference method → sample volume: 4 ml plasma
- QIAsymphony DSP Virus/Pathogen Midi Kit with "virus cell-free 1000" protocol (VCF1000) → sample volume: 1 ml plasma
- QIAsymphony DSP Virus/Pathogen Midi Kit with modified 4 ml protocol (VCF4000) → sample volume: 4 ml plasma
- Circulating DNA yield determined by real-time PCR (18S coding sequence, 66 bp and 500 bp amplicons)

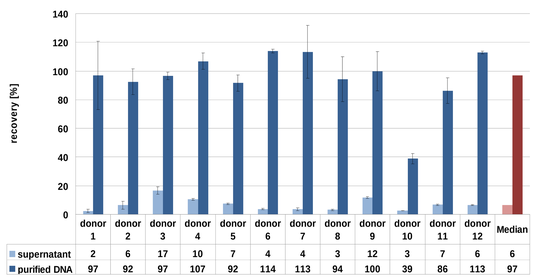
Results were calculated as target copies per ml plasma compared to the results obtained with the QIAamp Circulating Nucleic Acid Kit.

Modified 4 ml Protocol: Mean, Median Yields

ccfDNA was analyzed in purified total circulating nucleic acids from plasma as described in the left panel. Circulating DNA yield was quantified by duplex real-time PCR specific for 18S rRNA coding sequences. Two targets (66 bp and 500 bp) were co-amplified. The ccfDNA yields were calculated as target copies per ml plasma (upper panel) and are shown as relative recovery compared to the QIAamp Circulating Nucleic Acid Kit as reference (= 100%).

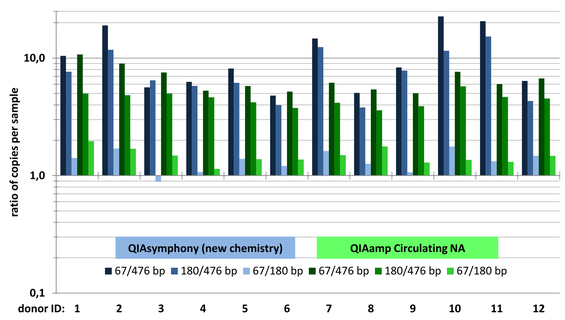


Results: Novel Extraction Chemistry – ccfDNA Yields



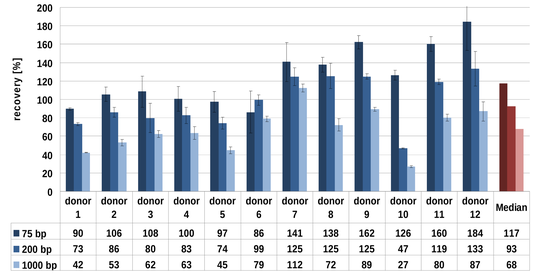
Circulating cell-free DNA was purified from 5 ml plasma from 12 individual donors using the novel enrichment protocol on the QIAsymphony SP instrument (elution in 150 µl). DNA yield was quantified by real-time PCR targeting a 66 bp amplicon within the 18S ribosomal RNA coding sequence using the QIAGEN QuantiTect® Multiplex PCR Kit. The QIAamp® Circulating Nucleic Acid Kit (QIAGEN) served as reference method (=100% recovery) and was also used for the purification of any residual ccfDNA from the supernatant after binding of ccfDNA to magnetic beads.

Results: Novel Extraction Chemistry – ccfDNA Size



Circulating cell-free DNA was purified from 5 ml plasma from 12 individual donors using the novel enrichment protocol on the QIAsymphony SP instrument (elution in 150 µl). DNA yield was quantified by APP qPCR using four amplicons as described in the panel above. The copy number ratios obtained with the APP qPCR assay were calculated to track the DNA fragment size-dependent ccfDNA extraction efficiency.

Results: Novel Extraction Chemistry – Spike-in Control



In order to track a possible size-selectivity of the procedure and as internal control, DNA fragments (75 bp, 200 bp, 1000 bp) were added to plasma samples at 200,000 copies/sample. Circulating cell-free DNA was purified from 5 ml plasma of 12 individual donors using the novel enrichment protocol on the QIAsymphony SP instrument (elution in 150 ml). DNA yield was quantified by triplex, real-time PCR targeting regions within the 75 bp, 200 bp and 1000 bp fragment using the QIAGEN QuantiTect® Multiplex PCR Kit. The QIAamp® Circulating Nucleic Acid Kit (QIAGEN) served as reference method (=100%).

Summary

- The efficiency of circulating, cell-free DNA (ccfDNA) extraction was similar using both new automated QIAsymphony protocols compared to the QIAamp Circulating Nucleic Acid Kit.
- Fully automated ccfDNA extraction —including fetal DNA & RNA— from up to 4 ml plasma can be performed on the QIAsymphony® SP using the modified QIAsymphony protocol in combination with the QIAsymphony DSP Virus/Pathogen Midi Kit (with up to 24 samples per QIAsymphony® run).
- The novel QIAsymphony extraction chemistry (in development) enables automated ccfDNA recovery, including fetal ccfDNA, from up to 5 ml plasma up to 96 samples per QIAsymphony® SP run). Short ccfDNA fragments are recovered efficiently.

Parts of this work have received funding from the European Union Seventh Framework Programme (FP7) under grant agreement no 222916 (Project SPIDIA*).

* SPIDIA = Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics | www.spidia.eu

Sample & Assay Technologies