

Title:

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

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Objective:

Circulating, cell-free DNA (ccfDNA), present in blood plasma, has become a crucial analyte for non-invasive prenatal diagnostics (NIPD) and cancer biomarker analysis. 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.

Methods:

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.

Results:

The novel protocol (a) showed a median ccfDNA recovery of 97% (n=12; range: 39–114%) and the modified protocol (b) showed a median recovery of 126% (n=24; 87–222%) compared to the QIAamp CNA Kit. The median recoveries of spiked DNA fragments for protocol (a) were 117% for 75bp, 93% for 200bp, and 68% for 1000bp, compared to the reference method.

Conclusions:

The efficiency of ccfDNA extraction was similar using both new automated QIASymphony protocols compared to the QIAamp CNA Kit and the extracted ccfDNA showed no PCR inhibition. The novel protocol (a) enables automated ccfDNA recovery, including fetal ccfDNA, from up to 5 ml plasma (up to 96 samples per QIASymphony® SP run). The modified protocol (b) enables fully automated ccfDNA extraction from up to 4 ml plasma (up to 24 samples per QIASymphony® SP run).

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

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