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Data Article

Adjustments to the preanalytical phase of quantitative cell-free DNA analysis

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ABSTRACT

Evaluating the kinetics of cell-free DNA (cfDNA) in the blood of cancer patients could be a strong auxiliary component to the molecular characterization of cfDNA, but its potential clinical significance is obscured by the absence of an analytical consensus. To utilize quantitative cfDNA assessment with confidence, it is crucial that the preanalytical phase is standardized. In a previous publication, several preanalytical variables that may affect quantitative measurements of cfDNA were identified, and the most confounding variables were assessed further using the growth medium of cultured cancer cells as a source of cfDNA ("Cell-free DNA: Preanalytical variables" [1]). The data accompanying this report relates to these experiments, which includes numerous changes to the sample handling and isolation protocols, and can be used for the interpretation of these results and other similar experiments by different researchers.

Specifications table

Subject area	Biochemistry, molecular biology
More specific subject area	Clinical biochemistry, translational oncology, prenatal diagnostics

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Type of data	Excel spreadsheet, table
How data was acquired	PCR amplification of cell-free DNA was measured using a real-time quantitative assay for the β-globin gene. All assays were performed on a Rotor-Gene Q detection system (Qiagen) using a 72 well ring-setup.
Data format	Analyzed
Experimental factors	Centrifugation, medium storage temperature, medium thawing temperature, medium storage tube type, treatment with denaturing agents, combining snap freezing with proteinase K, binding buffer type, elution volume, elution regime and elution tube type.
Experimental features	Cell-free DNA was extracted directly from growth medium collected from 143B osteosarcoma cells in culture, and then quantified by real-time PCR. Several variations to the standard procedure were evaluated.
Data source location	South Africa
Data accessibility	The data is with this article

Value of the data

- This data will be useful considerations when optimizing protocols and setting up a standard operating procedure, which should expedite the translation of cfDNA analyses to clinical practice.
- This data could be compared to other studies that investigated the effect of methodological variables on quantitative measurements of cfDNA.
- This data could be used to interpret studies that investigated the effect of methodological variables on qualitative measurements of cfDNA.

1. Data

In order to investigate the effects of several adjustments to the preanalytical phase of quantitative cfDNA measurements, the growth medium of cultured cancer cells was used as a source of cfDNA. The data in this report was obtained by amplifying cfDNA with real-time PCR, after it had been extracted under different preanalytical conditions. The data is presented in a supplementary file as a single table, which includes several quantitative measurements of cfDNA following modifications to the standard protocol followed. These changes are described in Table 1.

2. Experimental design, materials and methods

2.1. Cell culturing

Culture medium of the human bone cancer (osteosarcoma) cell line 143B (ATCC® CRL-8303™) was used as a source of cfDNA. Given that DNA levels in growth medium fluctuate much like cfDNA in the blood of humans, we could use it as a model to evaluate the effect of different variables on both high and low concentrations of cfDNA. Cells were cultured in T75 flasks in Dulbecco's Modified Eagle's medium (DMEM) (HyClone; SH30243.01) supplemented with 10% fetal bovine serum (Biochrom; S0615) and 1% penicillin/streptomycin (Lonza; DE17-602E) at 37 °C in humidified air and 5% CO_2. After the cells have reached the necessary confluency, the culture medium was removed, processed and stored at −80 °C in 15 ml tubes.