Effects of Blood-Processing Protocols on Cell-free DNA Quantification in Plasma

To the Editor:

Recently, qualitative analysis of cell-free DNA in blood plasma has attracted much interest for the diagnosis of cancer, fetal gender, Rh+ status, and inherited disorders (1, 2). Other studies have shown that quantification of total plasma DNA may indicate fetal chromosomal aneuploidies and pregnancy-associated complications or the presence/recurrence of cancer (1, 3, 4). Irrespective of the type of study (qualitative or quantitative DNA analysis), it is important that cell-free plasma DNA is not contaminated with cellular DNA that interferes with analysis and accurate quantification.

To prevent cellular DNA contamination, Chiu et al. (5) emphasized the need of preanalytical standardization of blood-processing protocols. The authors note that after centrifugation of blood at a low speed (800g), the amount of isolated DNA from plasma is affected by the presence of cells that remain in the plasma fraction. An additional centrifugation step (16,000g) or filtering of the plasma is necessary to produce absolutely cell-free plasma DNA. Because most laboratories, including ours, centrifuge blood at relatively low speeds (800–1500g) and store plasma without additional treatment, these results may have a serious impact on the usefulness of previously collected plasma samples for retrospective DNA analysis/quantification.

To confirm the results of Chiu et al. (5), we collected EDTA blood in 7-mL Vacutainer Tubes from 18 healthy women with uncomplicated pregnancies, attending the outpatient clinic of the University Medical Centre Nijmegen, after 21 weeks of gestation. Blood was centrifuged at 800g for 10 min. The plasma supernatant was carefully removed to ~0.5 cm above the buffy coat layer, homogenized, and divided into two portions: fractions A (400 μL) and B...
Fraction B was centrifuged additionally at 16 000g for 5 min, and 400 μL of the supernatant was transferred to a new tube. All fractions were processed within 3 h after collection and stored at −20 °C for at least 1 month. After thawing, DNA in the fractions was extracted exactly according to the protocol of Chiu et al. (5). Both fractions A and B from each pregnant woman were processed in the same batches during the isolation and quantification procedures. Copies of the albumin gene were quantified with real-time PCR and transformed to cell-equivalents/mL of plasma (CE/mL), as described previously (3).

The quantification results for plasma fractions A and B from 18 pregnant women are shown in Fig. 1A. Although the median number of cell-equivalents in fractions B (422 CE/mL) correlated well with the results of Chiu et al. (5), surprisingly we detected no significant amounts of additional DNA (from cellular origin) in fractions A. Obviously, in our samples, all cellular DNA had been removed from the plasma by centrifugation at 800g. After careful reanalysis of the protocol of Chiu et al., we could identify only a single difference with our procedure: we collected blood in 7-mL EDTA collection tubes instead of 10-mL tubes.

To demonstrate the possible effect of this difference, we performed an additional experiment in which we collected blood from 10 nonpregnant donors into both 10- and 7-mL tubes. Blood and plasma were processed as described before. In these samples, after DNA isolation, contaminating cellular DNA was still present in the plasma fraction of the 10-mL collection tubes after centrifugation at 800g (Fig. 1B, fraction A). It was removed after additional centrifugation at 16 000g, confirming the results of Chiu et al. (Fig. 1B, fraction B). Moreover, plasma DNA concentrations derived from the plasma from 7-mL tubes (Fig. 1B, fraction D) were identical to the DNA concentrations in cell-free plasma (Fig. 1B, fraction B), corroborating our previous results shown in Fig. 1A.

From these results, we concluded that the amount of blood collected (i.e., the size of the blood collection tube) affects the presence of contaminating cells in the plasma fraction. Because these cells were present in plasma both from pregnant women (5) and nonpregnant individuals (our results), leukocytes are the most likely explanation for the contaminating cells in the 10-mL blood collection tubes.

In parallel, we questioned whether these contaminating cells in plasma (Fig. 1B, fraction A) could be removed after storage at −20 °C. We hypothesized that as a result of the freeze-thaw step, contaminating cells lyse, but their nuclei remain intact and can still be eliminated by high-speed centrifugation. Indeed, high-speed centrifugation of fraction A plasma samples after storage completely eliminated the cellular contamination (Fig. 1B, fraction C). The efficiency of elimination was identical to that of high-speed centrifugation before storage (Fig. 1B, fraction B).

We conclude that the efficiency of removal of contaminating cellular DNA from plasma is dependent on many preanalytical factors, including centrifugal force, amount of collected blood, and pipetting efficacy. Most importantly, we have shown that irrespective of the protocol used for plasma collection, contaminating cells that remained in the plasma can be removed after storage (−20 °C) of samples. Therefore, frozen plasma collections can be used retrospectively for DNA analysis when subjected to additional centrifugation at 16 000g after thawing.

References

Dorine W. Swinkels1* Erwin Wiegertink1 Eric A.P. Steegers2 Jacques B. de Kok1

1 Department of Clinical Chemistry University Medical Centre Nijmegen 6500 HB Nijmegen, The Netherlands
2 Department of Obstetrics and Gynaecology University Hospital Rotterdam 3000 CA Rotterdam, The Netherlands

*Address correspondence to this author: Department of Clinical Chemistry/564, University Medical Centre Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Fax 31-243541743; e-mail D.Swinkels@umcn.nl.