

Preservation and Amplification of Fetal Cell-Free DNA in Maternal Plasma for Noninvasive Prenatal Diagnosis

M. R. Fernando,¹ K. Chen¹, S. Norton¹, W. L. Ryan¹, and C. Bassett,²

¹Research and Development Division, Streck, Inc., La Vista, NE 68128, ²Women's Center, Methodist Physicians Clinic, West Dodge Road, Omaha, NE, USA

Streck[®]
Omaha, NE 68128

Abstract

Background

Even though the existence of cell-free DNA in blood plasma was discovered much earlier, its usefulness in disease diagnosis and prognosis was not very well understood until 1977 when Leon et al. (1) demonstrated that cell-free plasma DNA is elevated in cancer patients. After this initial observation, cell-free DNA in the plasma where it is free of cellular genomic DNA, has been examined by many (2) for possible uses in cancer diagnosis as well as many other potential diagnostic uses. The discovery of the existence of circulating cell-free fetal DNA in maternal plasma by Lo et al. in 1997 was a new milestone in non-invasive prenatal diagnosis (3). This methodology can provide the much needed fetal nucleic acids for crucial prenatal diagnosis by a simple blood draw from the mother. A number of studies (4) describe the importance of getting cell-free plasma DNA devoid of cellular DNA for this purpose. The usual methods involve careful centrifugation and handling of the blood. Here, we describe an alternative method that provides cell free DNA from maternal blood which utilizes a vacuum collection tube containing stabilizing agents and nuclease inhibitors to obtain cell-free plasma DNA free from post-sampling contamination with cellular genomic DNA.

Objective

To develop a standardized blood collection device that preserves fetal cell-free DNA from nuclease mediated degradation and minimizes maternal cell-free DNA background in maternal plasma at room temperature.

Methods

Blood samples were drawn from healthy pregnant donors into regular K-EDTA and Streck-developed Cell-Free DNA BCT (blood collection tubes) and kept at room temperature until further processing at different time points. Plasma was separated by centrifugation, the cell-free DNA extracted and then the total and fetal cell-free DNA in plasma was quantified by quantitative real time PCR using the hypermethylated RASSF1A gene promoter region as an indicator for fetal cell-free DNA.

Results

Maternal blood drawn into Streck's Cell-Free DNA BCT showed no change in the relative percentage of fetal cell-free DNA during 14 days of storage at room temperature, whereas blood drawn into regular K-EDTA tubes showed a reduction in the relative percentage of fetal cell-free DNA over the time period. Cell-free DNA extracted from blood stored in Cell-Free DNA BCT for 14 days was amplified by whole genome amplification which resulted in at least a 80-fold increase in fetal cell-free DNA in maternal plasma.

Conclusions

Using Streck's Cell-Free DNA BCT, it is possible to preserve fetal cell-free DNA for extended times as well as minimize post sampling maternal Cell-free DNA background which helps to keep the relative percentage of fetal cell-free DNA in maternal blood unchanged. Fetal cell-free DNA so preserved can be amplified by whole genome amplification technology, thus providing the fetal nucleic acids as a starting material for nucleic acid based prenatal diagnostic tests.

References

1. Leon SA, Shapiro B, Sklaroff DM, and Yaros MJ. Cancer Research 1977; 37: 646-650.
2. Fleischhacker M. and Schmidt B. Biochimica et Biophysica Acta 2007; 1775: 181-232.
3. Lo YM, Corbetta N, Chamberlain PF, et al. Lancet 1997; 350:485-487.
4. Chiu RW, Poon LL, Lau TK, Leung TN, Wong EM, Lo YMD. Clin Chem 2001; 47: 1607-1613.

Introduction

Even though the existence of cell-free DNA in blood plasma was discovered much earlier, its usefulness in disease diagnosis and prognosis was not very well understood until 1977 when Leon et al. (1) demonstrated that cell-free plasma DNA is elevated in cancer patients. After this initial observation, cell-free DNA in the plasma, free of cellular genomic DNA, has been examined by many (2) for possible use in cancer diagnosis and many other potential diagnostic uses. The discovery of the existence of circulating cell-free fetal DNA in maternal plasma by Lo et al. in 1997 was a new milestone in non-invasive prenatal diagnosis (3). This can provide the much needed fetal nucleic acids for non-invasive prenatal diagnosis by a simple blood draw from the mother. A number of studies (4) describe the importance of getting cell-free plasma DNA devoid of cellular DNA for this purpose. The usual methods involve careful centrifugation and handling of the blood. We describe an alternative method that provides cell free DNA from maternal blood which utilizes a vacuum collection tube containing stabilizing agents and nuclease inhibitors to obtain cell-free plasma DNA free from post-sampling contamination with cellular genomic DNA.

Results

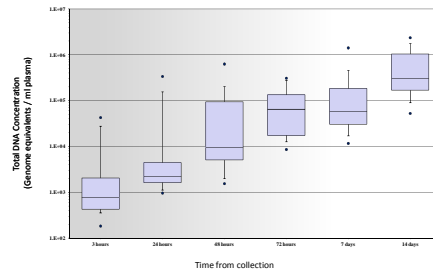


Figure 1. Total plasma cell-free DNA concentration in first trimester maternal blood samples drawn into regular EDTA blood collection tubes and stored at 22°C.

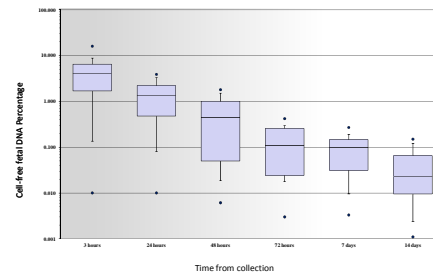


Figure 3. Cell-free fetal plasma DNA concentration in first trimester maternal blood samples drawn into regular EDTA blood collection tubes and stored at 22°C.

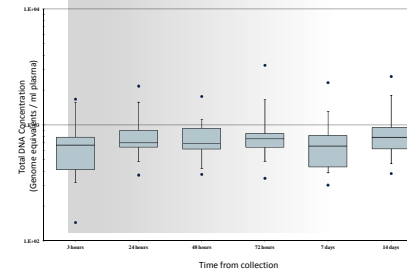


Figure 2. Total plasma cell-free DNA concentration in first trimester maternal blood samples drawn into Streck Cell-Free DNA BCT and stored at 22°C.

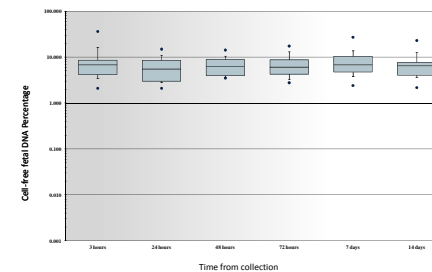


Figure 4. Cell-free fetal plasma DNA concentration in first trimester maternal blood samples drawn into Streck Cell-Free DNA BCT and stored at 22°C.

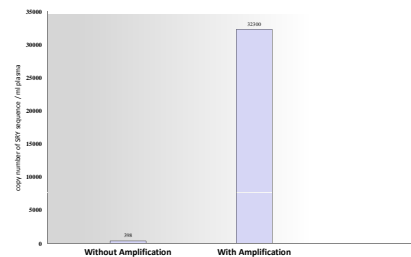


Figure 5. Amplification of fetal cell-free DNA in Maternal Blood Stored in Streck Cell-Free DNA BCT for 14 Days by Whole Genome Amplification. Y chromosome SRY region sequence was used as an indicator of fetal cell-free plasma DNA.

Materials and Methods

Pregnant donor recruitment

Pregnant donors were recruited from Methodist Hospital, Omaha NE, USA. This study was approved by the institutional review board of the Methodist Hospital, Omaha NE and informed consent was obtained from all donors prior to blood draw.

Blood collection

Two 10 ml blood samples were drawn into two different blood collection tubes from each donor. One tube was a regular K₂EDTA blood collection tube (BD vacutainer) and the other was a vacuum blood collection tube developed by Streck Inc. (Cell-Free DNA BCT) which contained chemical compounds in order to stabilize nucleated blood cells and inhibit plasma nucleases. Blood was mixed well immediately after drawing into the tubes by inverting the tube several times.

Sample processing

Aliquots (1.5 ml) of blood were removed from each tube at 3 hours, 24 hours, 48 hours, 72 hours, 7 days and 14 days. Blood samples were centrifuged at room temperature at 1600 g for 10 minutes. The upper plasma layer was carefully removed without disturbing the buffy coat and transferred to a new tube using a pasteur pipet and then centrifuged at 16000 g at room temperature for another 10 minutes. The cell-free plasma was carefully removed from the tube without disturbing the cell pellet and transferred into a new tube.

Plasma cell-free DNA isolation

Cell-free plasma DNA was purified using a commercially available NucleoSpin[®] Plasma XS kit (MACHEREY-NAGEL Inc., Bethlehem, PA).

Real Time quantitative PCR

Primers and probes for the real time PCR quantification of β -actin and RASSF1A sequences were prepared as described by Chan et al. (5). Primers for the Y-chromosomal sex determining region (SRY) were prepared as previously described (6).

Conclusions

Using Streck's Cell-Free DNA BCT, it is possible to preserve fetal cell-free DNA for extended times as well as minimize post sampling maternal cell-free DNA background. This helps to keep the relative percentage of fetal cell-free DNA in maternal blood unchanged. Fetal cell-free DNA so preserved can be amplified by whole genome amplification technology providing the fetal nucleic acids as the starting material for nucleic acid based prenatal diagnostic tests.

References

1. Leon SA, Shapiro B, Sklaroff DM, and Yaros MJ. Cancer Research 1977; 37: 646-650.
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3. Lo YM, Corbetta N, Chamberlain PF, et al. Lancet 1997; 350: 485-487.
4. Chiu RW, Poon LL, Lau TK, Leung TN, Wong EM, Lo YMD. Clin Chem 2001; 47: 1607-1613.
5. Chan KCA, Ding C, Gerovassili A, et al. Clin Chem 2006; 52: 2211-2218.
6. Lee T-H, Paglieroni T, Ohto H, et al. 1999. Blood 1999; 93: 3127-3139.

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¹Research and Development Division, Streck, Inc., La Vista, NE 68128.

²Women's Center, Methodist Physicians Clinic, West Dodge Road, Omaha, NE, USA

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