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Optimizing blood collection, transport and storage conditions for cell free DNA increases access to prenatal diagnostic testing☆

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ABSTRACT

Objectives: Fetal mutations and fetal chromosomal abnormalities can be detected by molecular analysis of circulating cell free fetal DNA (ccffDNA) from maternal plasma. This comprehensive study was aimed to investigate and verify blood collection and shipping conditions that enable Noninvasive Prenatal Diagnostic Testing. Specifically, the impact of shipping and storage on the stability and concentration of circulating cell-free DNA (ccfDNA) of blood in Streck® Cell-Free DNA™ Blood Collection Tubes (Streck BCTs, Streck, Omaha NE), designed to minimize cellular degradation, and thus effectively prevent dilution of ccffDNA by maternal genomic DNA, was evaluated.

Design and methods: Peripheral venous maternal blood was collected into Streck BCTs to investigate four aspects of handling and processing conditions: (1) time from blood draw to plasma processing; (2) storage temperature; (3) mechanical stress; and (4) lot-to-lot tube variations.

Results: Maternal blood stored in Streck BCTs for up to 7 days at ambient temperature provides stable concentrations of ccffDNA. The amount of fetal DNA did not change over a broad range of storage temperatures (4 °C, 23 °C, 37 °C, 40 °C), but the amount of total (largely maternal) DNA increased in samples stored at 23 °C and above, indicating maternal cell degradation at elevated temperatures. Shipping maternal blood in Streck BCTs, did not affect sample quality.

Conclusions: Maternal plasma DNA stabilized for 0 to 7 days in Streck BCTs can be used for non-invasive prenatal molecular applications, when temperatures are maintained within the broad parameters assessed in this study.

Introduction

Chromosomal, genetic and biochemical abnormalities of the fetus are routinely detected by analyzing fetal cells obtained through invasive procedures such as amniocentesis and chorionic villus sampling [1]. A small but definite risk of injury to both mother and fetus is imparted by invasive sampling procedures [2]. An alternate source of genetic material that accurately represents fetal status is circulating cell-free fetal DNA (ccffDNA) found in maternal plasma. The placenta is thought to be the main source for ccffDNA, and consequent clearance of ccffDNA from maternal plasma occurs within hours after birth [3,4]. Recently the use of ccffDNA has enabled the introduction of non-invasive prenatal testing (NIPT) methods such as fetal RHD genotyping from maternal plasma [5] and fetal aneuploidy detection in high risk women [6]. However, care must be taken to prevent an increase of circulating maternal DNA after phlebotomy (through maternal white cell lysis), because quantitative applications, such as non-invasive aneuploidy detection, are impaired by a relative decrease in fetal fraction (FF). Typical fetal fractions range from 2% to 40% with a mean of 10% of total ccfDNA across varying gestation ages [6,10]. The minimum fetal fraction for accurate determination of Trisomy 21 is 4% as measured by the Fetal Quantifier Assay (FQA) and set as QC cut-off [6]. Thus a small increase in maternal DNA could reduce the fetal fraction below the 4% QC cut off, particularly in cases of low initial fetal fraction. Below 4% FF no test results would be reported. Enabling the access of a broad population of mothers to non-invasive prenatal testing methods, requires robust and validated blood collection devices and processing protocols.

Abbreviations: ccf, circulating cell-free; ccffDNA, circulating cell-free fetal DNA; BCT, blood collection tube; NIPT, non-invasive prenatal testing; IRB, Institutional Review Board; FF, fetal fraction; FQA, Fetal Quantifier Assay; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometer; NSC, no storage control; MPS, massively parallel sequencing; NTC, no template control.

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To maintain high fetal fraction, processing protocols for maternal blood collected into standard EDTA Tubes require cold storage of the blood samples followed by plasma preparation within 6 h. Plasma preparation initiates with a low speed centrifugation of maternal blood for fractionation of the plasma from blood cells. The plasma layer is subsequently removed and centrifuged at a higher speed to pellet any residual debris from the plasma. Plasma processing is cumbersome and thus only performed at collection sites when absolutely necessary due to location or other circumstances. The requirement for immediate processing to plasma for blood collected in standard EDTA Tubes, and the associated costs of processing at collection sites, would unnecessarily limit the availability of NIPT to a broad population. To overcome these challenges, an ideal blood collection device would enable shipment of whole blood at ambient temperatures (6 °C–37 °C) and extend the timeframe for plasma processing. Such a device would facilitate centralized processing and analysis. Several alternatives exist to EDTA Tubes for collecting blood and preparing plasma for molecular diagnostic tests. Three potential tube types for maintaining fetal fractions can be found: tubes that are designed to create a physical barrier (gel plug or mechanical separator) between cellular and non-cellular blood components, tubes providing reagents for keeping maternal blood cells intact and active for a defined period of time, and tubes with cell-preserving reagents. A tube with cell-preserving reagents to prevent white blood cell degradation (which would release maternal DNA) and inhibit nuclease mediated DNA degradation for up to 14 days at ambient temperature was recently introduced by Streck and marketed as Streck Cell-Free DNA™ BCT (Streck BCTs)[7].

Barrett and colleagues revealed that the concentrations of "short-fragment" ccffDNA from blood collected Streck BCTs did not change when stored at ambient temperature over a period of 72 h. However, when blood was stored in EDTA Tubes for 72 h, an increase in "long-fragment" (maternal) plasma DNA was evident [8]. Results by Hidestrand et al. indicated that in blood collected into EDTA Tubes that were shipped either with or without frozen ice packs had an increased level of total plasma DNA after 72 h. When whole blood in Streck BCTs is shipped without ice packs (72 h) the level of total DNA is unchanged, while when shipped with frozen ice packs, the level of total DNA is reported to increase [9].

This study was aimed to characterize the ability of the Streck BCTs to maintain ccff DNA concentrations and inhibit nuclease mediated DNA degradation under conditions that are pragmatic for routine use for clinical applications. Our evaluation of the utility of the Streck BCT to preserve the integrity of the fetal DNA fraction focused on four key variables: influence of storage time (up to 14 days), storage temperature (for 24 h of storage), mechanical stress (ASTM Standard D5276-98) and lot-to-lot consistency (3 lots).

Materials, methods and experimental

Blood samples

Blood collection protocols

Patients were selected based on the following inclusion criteria: 1) Subject must be pregnant carrying a singleton fetus of 10–16 weeks of gestational age inclusive. 2) Subject must be 18 years of age or older or an emancipated minor. 3) Subject has consented to have up to 50 mL of whole blood collected at one or more monthly (≥25 days) scheduled clinic visits.

Blood from patients, up to 5 × 10 mL of peripheral blood, was collected by venipuncture in varying combinations of two types of evacuated blood collection tubes, Vacutainers® – K_2EDTA Tubes (BD # 366643, referred to as EDTA Tubes in text; Becton, Dickinson and Company Franklin Lakes, NJ) and Cell-Free DNA Streck™ BCT (Streck # 218962, referred to as Streck BCTs in text; Streck Omaha, NE). Streck BCTs contain K_3EDTA as anticoagulant.

EDTA Tubes

Blood collected in K_2EDTA Tubes was transported chilled, with refrigerated ice packs, to a processing center and plasma was isolated within 6 h of blood draw unless otherwise indicated. The first centrifugation was conducted at 2500 ×*g* for 10 min at 4 °C, after which the plasma was carefully removed and transferred to a fresh tube. The second centrifugation was conducted at 15,500×*g* for 10 min at 4 °C. The resultant plasma was collected and stored frozen at ≤−70 °C until analysis.

Streck BCTs

Blood collected in Streck BCTs was shipped at ambient temperature to a processing center and plasma was isolated within 24 h of blood draw, unless otherwise indicated. The first centrifugation was at 1600 ×*g* for 15 min at room temperature, after which the plasma was carefully removed and transferred to a fresh tube. The second centrifugation was at 2500×*g* for 10 min at room temperature. The resultant plasma was collected and stored frozen at ≤−70 °C until analysis.

DNA extraction

EDTA and Streck BCT plasma samples were thawed and centrifuged at 1600 ×*g* for 10 min at 4 °C. Four mL of the supernatant was removed and circulating DNA was extracted with the QIAamp® DSP Circulating NA Kit (QIAGEN Cat# 61504, Hilden, Germany). The manufacturer's protocol was followed for EDTA plasma; Streck plasma extraction utilized an extended Proteinase K digestion time of 60 min. DNA was eluted with 55 μL of Qiagen Buffer AVE (elution buffer) from the QIAamp kit.

DNA quantitation

Fetal and total circulating DNA was detected using the FQA, a methylation-sensitive, single nucleotide extension MALDI-TOF MS assay [10]. This assay is capable of determining the gender of the fetus, and of quantifying fetal DNA (regardless of gender). FQA was run as indicated with 10 or 12.5 μL of extracted circulating DNA in quadruplicate PCR and quadruplicate MS reactions (16 reactions total). Data were analyzed as described [10]; total and fetal copies per mL plasma are provided by dedicated scripts, from which the fetal fraction was calculated. For total copies the dynamic range of the FQA assay is between 450 and 7500 [10]. Samples with total DNA copy numbers that were above the maximum dynamic range of the assay were adjusted to 7500 copies before normalization.

Study variables

Storage time

Blood was stored in EDTA Tubes and in Streck BCTs for 1 to 14 days and compared to EDTA or Streck no-storage-control (NSC), respectively. From each donor (20 pregnant female, 10 non-pregnant female, or 10 male subjects per time point, respectively) blood was collected into 2 EDTA Tubes and 2 Streck BCTs (10 mL per tube). EDTA NSC and Streck NSC were generated by processing blood into plasma within 6 h post blood draw. Blood in remaining EDTA Tubes and Streck BCTs was stored for either 1 day, 2 days, 3 days, 4 days, 7 days, or 14 days, after which plasma was prepared and stored frozen at ≤−70 °C until use.

Before extracting ccfDNA, all plasma samples were thawed and centrifuged at 1600 ×*g* for 10 min to pellet debris. FQA reactions were performed in quadruplicate using 10 μL of extracted DNA [10]. Data were analyzed using either: i) ANOVA for EDTA and Streck NSC for Day 1 to Day 14, and ii) comparing EDTA NSC of a specific subject (e.g. Subject 1 of Day 1) to the matched samples stored in EDTA Tubes and Streck BCTs.

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