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

David Wong [a], Sharmili Moturi [a], Vach Angkachatchai [a], Reinhold Mueller [a], Grace DeSantis [a], Dirk van den Boom [b], Mathias Ehrich [b,*]

[a] Sequenom Center for Molecular Medicine, 3595 John Hopkins Ct, San Diego, CA 92121, USA
[b] Sequenom Inc., 3595 John Hopkins Ct, San Diego, CA 92121, USA

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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 blood shipping conditions that enable Noninvasive Prenatal Testing. Specifically, the impact of shipping and storage on the stability and concentration of circulating cell-free DNA (ccfDNA) in Streck® Cell-Free DNA™ Blood Collection Tubes (Streck BCTs, Streck, Omaha NE). These BCTs were designed to minimize cellular degradation, and thus effectively prevent dilution of fetal ccf DNA 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 and genomic DNA release 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 gestational 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.

* Corresponding author at: Research & Development, Sequenom Inc., 3595 John Hopkins Ct, San Diego, CA 92121, United States.
E-mail address: mehrich@sequenom.com (M. Ehrich).

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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 to EDTA Tubes exist 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.

In analysis, median copy numbers for EDTA and for Streck Day 0 controls were calculated and used to normalize the values for Day 1, Day 2, Day 3, Day 4, Day 7 and Day 14 samples. To facilitate the comparison between the blocked time points, normalization was performed on the raw data. Normalization caused the resulting maximum values for total DNA copies on Days 7 and 14 total DNA to rise above 7500.

Constant temperature stress

Five aliquots of 10 mL of peripheral blood obtained from each of 20 pregnant females were drawn into separate Streck BCTs. One tube from each subject was processed into plasma within 30 h of blood draw (tube 1: NSC); the remaining 4 tubes were stored for 24 h at 4 °C, 23 °C (room temperature), 37 °C, and at 40 °C. After 24 h of incubation, blood was processed into plasma and frozen. For each subject a paired t-test was employed to compare each temperature point to the respective NSC.

Lot-to-lot consistency

Three different lots of Streck BCTs were tested in a paired set-up. Each lot was manufactured in separate production batches (different days). Two of the 3 lots were manufactured within a month of each other with the third manufactured 7 months earlier (based on expiration dates). Blood from each of 49 pregnant females was drawn into Streck BCTs lots 1, 2 and 3, resulting in 2 tubes of lot 1, 2 tubes of lot 2 and 1 tube of lot 3. ANOVA was applied. In addition the medians of each condition were analyzed using a paired t-test.

Massively parallel sequencing (MPS) library preparation

Blood from 22 subjects was collected into 2 EDTA Tubes and 2 Streck BCTs each. EDTA plasma was prepared within 6 h of blood draw, while Streck plasma was prepared after blood was stored for three days at room temperature. Circulating DNA was isolated and an aliquot was used in the Fetal Quantifier Assay for the evaluation of fetal fraction as an assessment of quality. Sequencing libraries were prepared according to a standard protocol [6]. Library concentrations were determined using a Caliper LabChip GX electrophoresis apparatus (Caliper BioSciences).

Statistical analysis

The R statistical programming environment was used to perform statistical analyses [11]. Additional analysis was performed using JMP 9 (Version 9.0.1, SAS).

Power analysis

Using results from previous paired studies, a power analysis in JMP was performed. Applying a power of 80%, $\alpha = 0.05$ on a sample size of 20, for fetal copy number and for fetal fraction (fetal copies/total copies per mL plasma), a 35% difference can be discerned; for total DNA copies the value drops to 30%. In the study comparing different production lots of Streck BCTs, the sample size was increased to 49 subjects, ensuring that potential differences in fetal copies of 22% can be reliably detected, 19% for total DNA copies and 21% for the fetal fraction.

Patients

Blood samples were collected under two separate Institutional Review Board (IRB) approved protocols, to ensure that enough samples were collected in the two week timeframe planned for the completion of blood collection for this study (both with Compass IRB). Informed consent was obtained from pregnant females, confirmed non-pregnant females, and males prior to collection of up to 5 × 10 mL of peripheral blood by venipuncture.

Results

After an initial screen of blood collection tubes for extended blood storage, including tubes with plugs, and with cell maintenance or cell

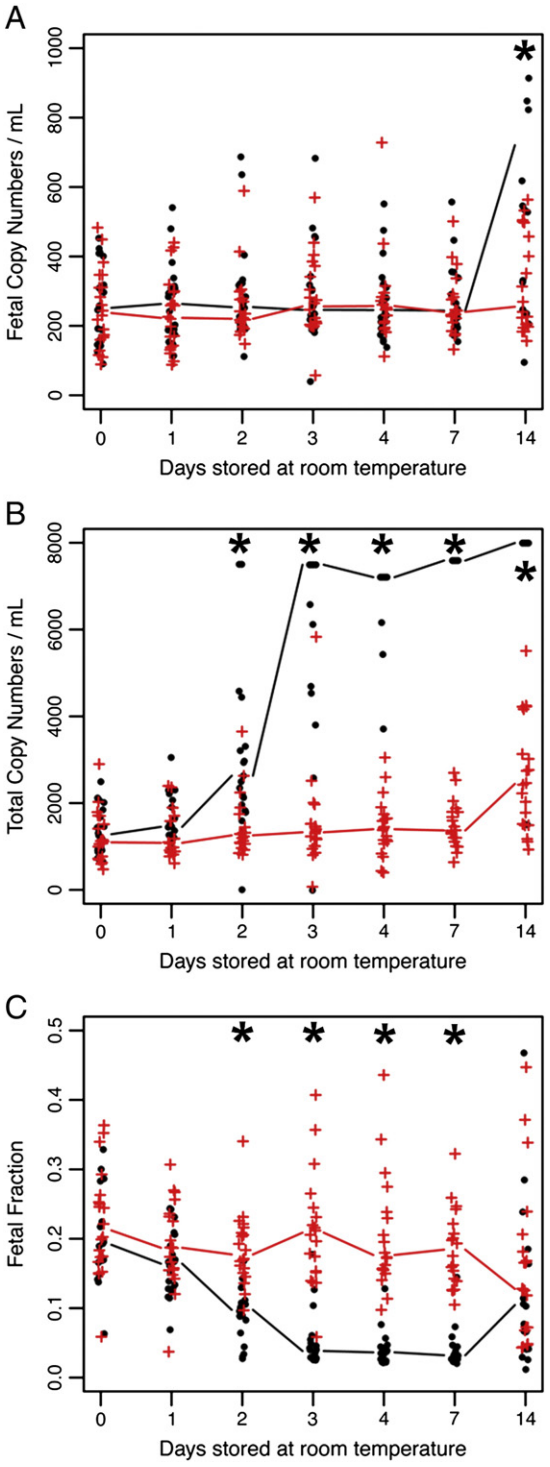

Fig. 1. Circulating DNA recovery from pregnant female blood stored for 0–14 days in control EDTA and cell preservation tubes. Results from 20 subjects per time point (Streck BCTs (red) and EDTA BCTs (black)) were normalized to their respective no storage controls. A. Fetal DNA, B. Total DNA and C. Fetal Fraction. Medians are connected by lines (red: Streck BCTs, black: EDTA Tubes). Statistically significant differences (p-value <0.05) between EDTA and Streck BCTs are indicated with an "*".

preserving reagents (data not shown), Streck BCTs were selected for further studies.

Analysis of 240 Streck plasma samples from individual pregnant women identified 42% female fetuses (n = 101) and 58% male fetuses (n = 139). Of the 240 pregnant females, gestational age was available in 218. The mean (median) gestational age was 14.73 (13.40) weeks, with a minimum of 10 and a maximum of 27 weeks. The range of the collected ccfDNA spanned 2% to 62%, 38–949 fetal copies/mL, and 154 to 7500 total copies/mL.

Effect of storage time on pregnant female whole blood in cell preservation tubes

The stability of ccfDNA in Streck BCTs was evaluated by comparing to standard EDTA Tubes. Four aliquots were analyzed for each time point: 2 aliquots, one in Streck BCT and one in EDTA Tubes were processed to plasma directly after blood collection. Two additional aliquots, again one in Streck BCT and one in EDTA Tubes, were processed after a predefined storage time. Using whole blood collected from 20 different pregnant women, six storage time points were evaluated, each with a corresponding no storage (Day 0) control. Total and fetal copy numbers were assessed by FQA and the raw data is shown in Supplemental Data Fig. 3. To enable a direct comparison between storage times (Day 1 to Day 14), a normalization schema was applied using the median of the no storage controls as a reference. A more detailed description of the normalization methods is provided in the Supplemental Data. The normalized data and analysis is presented in Fig. 1 and Table 1, along with the Day 0 control reference.

Whole blood collected and stored in EDTA Tubes prior to processing plasma demonstrates a statistically significant increase in total DNA copy numbers after only one day of ambient temperature storage. In EDTA Tubes, the increase in total copy number continued throughout 14 days of storage. By Day 2, an increasing amount of samples was measured outside the linear range for quantification (7500 copies). The EDTA median total copy numbers were 1454 for Day 1, 2631 for Day 2, 7494 for Day 3, 7207 for Day 4, 7590 for Day 7 and 7995 for Day14 (black line, Fig. 1A and Table 1). For blood collected in Streck BCTs the results are remarkably different. In Streck BCTs, at up to 7 days storage of whole blood, total copy numbers were not different from the immediately processed samples ($p > 0.06$, paired t-test, see Table 1). In Streck BCTs, at 14 days a statistically significant increase in total DNA copy numbers was observed (median = 2443, $p < 0.001$ at Day 14; red line, Fig. 1B).

Fetal copies were stable over 14 days for both EDTA and Streck plasma with one exception. The samples stored in EDTA for 14 days also showed an increase in fetal copy numbers (black line, Fig. 1A). This effect is most likely attributed to the extremely high total copy numbers, which led to incomplete digestion during the methylation sensitive restriction digest step in the assay (FQA).

Fetal fraction is calculated as the quotient of fetal copy numbers and total copy numbers. In this storage time study the fetal copy numbers remained mostly stable and consequently the fetal fraction is largely a reflection of the changes in total copy numbers. No statistically significant differences ($p > 0.163$, paired t-test) were observed for samples collected in Streck BCTs up to and including Day 7, while samples stored in EDTA Tubes demonstrated a significant decrease starting at Day 2 (Fig. 1C, Table 1).

Effect of storage time on non-pregnant female and male whole blood in cell preservation tubes

Results observed in samples from pregnant females were confirmed in non-pregnant female and male subjects. Analogous to the previous study, 10 subjects were enrolled per time point. The data do not show a statistically significant increase ($p > 0.071$) in total copy numbers for samples collected in Streck BCTs; however, starting on Day 2, total DNA is increased for samples stored in EDTA (Supplemental Data Figs. 4 and 5).

Effect of four different storage temperatures on pregnant female blood

Blood collected and shipped in Streck BCTs was stored at four temperatures. One tube each from 20 pregnant females was processed into plasma after it was received from the blood collection sites, usually within 30 h after blood draw (reference control sample). The remaining four blood tubes were stored for 24 h at 4 °C, 23 °C, 37 °C and 40 °C.

A paired t-test was employed to compare each temperature point to the reference control sample. Total DNA copies increased slightly after storage at 4 °C (median = 1174, $p = 0.343$), but a statistically significant effect was observed after storage at 23 °C (median = 1394, <0.001; Fig. 2B). A further increase in total copies was evident after storage at 37 °C (median = 1882, $p < 0.001$) and 40 °C (median = 1950, $p < 0.001$), leading to a continuous drop in fetal fraction. Fetal copy numbers at 4 °C, 23 °C, 37 °C and 40 °C were similar to the control (overall median = 166, Fig. 2A). Paired t-test results further revealed that the fetal fraction for samples stored at 23 °C (median = 0.115, $p < 0.001$) and the 37 °C (median = 0.08, $p < 0.001$, Fig. 2C) samples was significantly lower than the control samples.

Table 1
Median total, fetal copy numbers and fetal fraction in plasma from 20 pregnant females per time point, stored for 1 to 14 days in Streck BCTs and EDTA Tubes.

Median for pregnant female samples				p-values for paired t-test*			
	Fetal copies/mL	Total copies/mL	Fetal fraction		Fetal copies/mL	Total copies/mL	Fetal fraction
Streck BCTs							
Day 1	223	1085	0.187	Day 1	0.6537	0.686	0.1626
Day 2	221	1257	0.177	Day 2	0.5493	0.2628	0.2213
Day 3	256	1328	0.209	Day 3	0.9792	0.5333	0.8861
Day 4	257	1398	0.177	Day 4	0.3443	0.1165	0.4333
Day 7	241	1370	0.184	Day 7	0.8853	0.0612	0.3828
Day 14	255	2443	0.128	Day 14	0.5078	0.0001*	0.0050*
EDTA Tubes							
Day 1	263	1454	0.164	Day 1	0.9681	0.1487	0.0682
Day 2	254	2631	0.098	Day 2	0.9845	0.0007*	<0.0001*
Day 3	246	7494	0.038	Day 3	0.8693	<0.0001*	<0.0001*
Day 4	246	7207	0.036	Day 4	0.7667	<0.0001*	0.0261*
Day 7	244	7590	0.032	Day 7	0.7401	<0.0001*	<0.0001*
Day 14	721	7995	0.105	Day 14	0.0142*	<0.0001*	0.9806

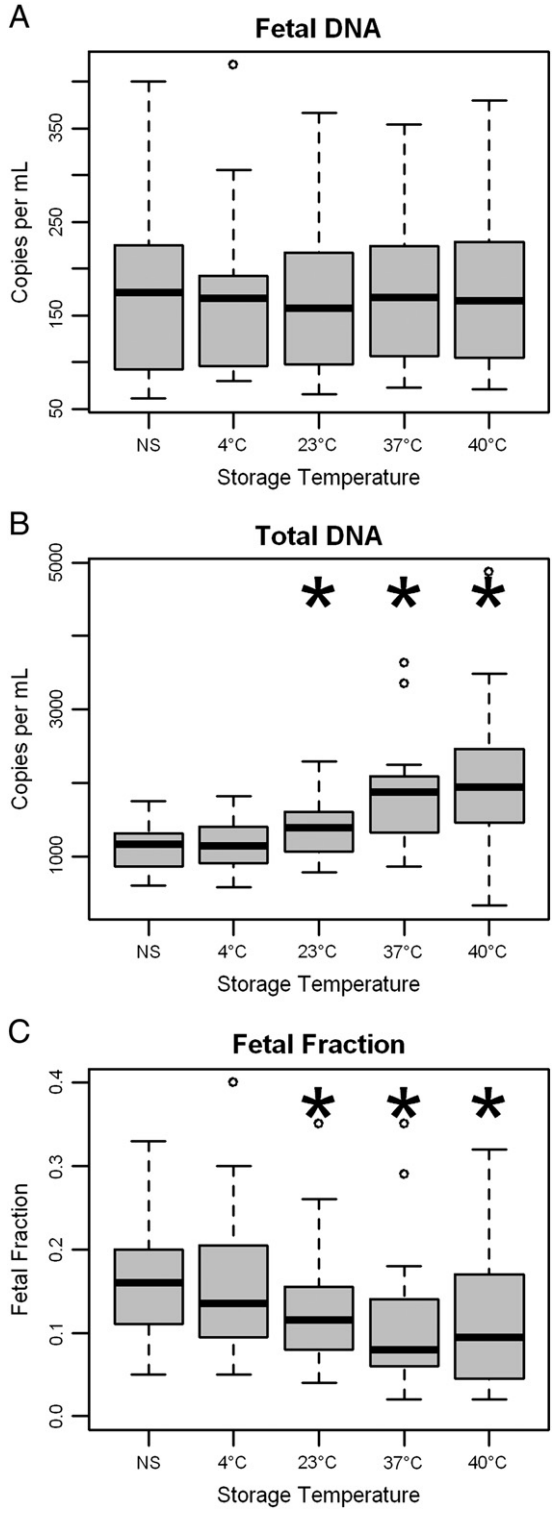

Fig. 2. Recovery of circulating DNA from pregnant female blood stored in cell preservation tubes 4 °C, 23 °C, 37 °C or at 40 °C. Blood stored in Streck BCTs for 24 h at the indicated temperatures before DNA extraction. A. Fetal DNA, B. Total DNA and C. Fetal Fraction. NS: no storage. Statistically significant differences (p-value <0.05) between EDTA and Streck BCTs are indicated with an "*".

Drop test for pregnant female blood in cell preservation tubes

To further investigate impact of shipping conditions and potentially rough handling on whole blood, a drop test according to ASTM Standard was employed. In this study, blood tubes were packaged with considerations to International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) regulations for the safe transport of Biological Substances, Category B (UN3373). Two tubes from each of 20 patients collected in Streck BCTs were packaged into an absorbent protective sleeve along with 3 additional Streck BCTs filled with water, to simulate a shipment. The tubes in sleeves were rolled up and placed into a standard biohazard bag. The bag was placed between insulating gel packages in 9 in. by 7 in. by 4.5 in. shipping box, which was sealed and dropped from exactly 3 ft above the floor. The two remaining non-drop aliquots from each patient served as controls. All dropped and control samples were unpacked and analyzed according to the standard protocol. This test was repeated with a fresh set of material (sleeves, biohazard bag, additional water filled Streck BCTs and shipping box) for each of the 20 patients used for this study. Based on a paired t-test, no statistically significant differences ($p > 0.181$) between dropped and control samples were observed for total or fetal DNA as well as fetal DNA percentage (Supplemental Data Fig. 7).

Cell preservation tube lot-to-lot consistency

In a paired set-up reproducibility in recovering circulating fetal DNA was compared in three production lots of Streck BCTs (Supplemental Data Fig. 8). ANOVA ($p = 0.1685$ for total copies, $p = 0.9080$ for fetal copies, $p = 0.1436$ for fetal fraction) showed no statistically significant differences between the three lots. A paired t-test showed a small but statistically significant difference in total circulating plasma DNA between lot 1 and lot 2 or lot 3 (Supplemental Data Table 3).

Massively parallel sequencing (MPS) library preparation with ccfDNA

Streck BCTs contain 200 μL of a proprietary preserving reagent. Previous studies have indicated that this reagent does not interfere with real time PCR results. EDTA and Streck ccfDNA was compared in a second downstream application, the library preparation for non-invasive prenatal aneuploidy detection by MPS. No significant difference in the DNA concentration of sequencing libraries was detected (EDTA mean = 171 nM and Streck mean = 164 nM, $p = 0.524$; Supplemental Data Fig. 9).

Discussion

This comprehensive study evaluates the operating range for a blood collection tube with cell stabilizing agents reported to prevent cell degradation. The stability of maternal blood collected in Streck BCTs for up to 14 days at ambient temperature was tested. For whole blood collected in Streck BCTs and stored at ambient temperatures over a period of 14 days, the concentration of fetal DNA did not significantly change. An increase in total DNA is noted only at Day 14 and this is likely caused by white blood cell degradation. The shipping and storage temperature has a larger than expected effect on cell integrity, with a strong temperature effect observed at 37 °C and 40 °C. Overall, when compared to standard EDTA Tubes the Streck BCTs show a clear effect of stabilizing cell integrity and fetal fraction and can thus enable a simplified sample collection process.

Currently the two main applications for plasma ccfDNA include cancer and prenatal diagnoses. The majority of these tests are run in centralized facilities similar to a core lab of a major hospital. Given the excellent shipping infrastructure in the United States it is safe to assume that samples can be transferred from the time of blood collection to the testing facility within 3 days throughout the continent using commercial carriers. The data presented in this study indicate that cell integrity is preserved for up to seven days. Besides shipping or storage times affecting the integrity of maternal cells, shipping temperature or physical shock might also be negative effectors. During shipping samples are likely to be exposed to a broad range of seasonally and geographically different temperatures. To investigate the impact of various temperatures a study was performed in which the

samples were stored at 4 °C, 23 °C, 37 °C and 40 °C. The results confirmed the impact of constant, higher temperatures on maternal circulating DNA concentrations. Temperature cycling between 6 °C and 37 °C also had an impact on total plasma DNA concentration and fetal fraction. However, with the experimental set-up shown here, the effects of cycling and increased temperature cannot be distinguished (Supplemental Data Fig. 6). Most carriers offer a limited form of temperature control during shipment of clinical samples. Based on our data, reported herein, a shipping container that provides some insulation to prevent exposure to temperatures >23 °C for prolonged periods of time is recommended for the transport of blood collected into Streck BCTs. Finally, a 3-foot drop of whole blood samples did not alter the circulating DNA concentration.

An interesting observation in this study is the stability of the results for fetal DNA contributions in the maternal plasma. Similar levels of fetal DNA were found in all experimental conditions in this study. The only increase of fetal DNA copies was observed after 14 days of storage in EDTA Tubes and is likely related to limitations of the measurement method. In the assay used here, unmethylated maternal DNA is digested with methylation-sensitive restriction enzymes and therefore not available for marker amplification [10]. When the amount of maternal DNA vastly exceeds fetal DNA (>1000 fold) incomplete digestion can lead to an overestimation of "fetal" DNA.

The need for a cell stabilizing BCT will be dictated by the analytical needs of the application. Qualitative applications, such as prenatal fetal RHD genotyping, are unlikely to require extended stabilization. Amplification of the RHD locus is specific and maternal DNA background in a phenotypically RHD negative mother is unlikely to interfere. In contrast, applications that require a quantitative assessment of fetal DNA, such as aneuploidy detection, are critically dependent on a low maternal background. In those applications, a BCT that prevents further maternal cell degradation can eliminate the need for a complex network of plasma processing sites across the country. As a result a broad adaptation of otherwise cost prohibitive collection methods can be enabled.

Conclusions

In summary, the results of the evaluation of Streck Blood Collection Tubes demonstrate that these tubes provide an effective alternative to blood collection in EDTA Tubes. Streck BCTs enable ambient shipment to a processing site up to 7 days after blood collection. Therefore these tubes and validated protocols for their use and handling can enable implementation of complex molecular testing of ccffDNA and thus increase patient access to safer non-invasive prenatal testing (NIPT) methods.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2013.04.023.

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