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Comparison of cell stabilizing blood collection tubes for circulating plasma tumor DNA

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Abstract

Objectives—Circulating plasma DNA is being increasingly used for biomedical and clinical research as a substrate for genetic testing. However, cell lysis can occur hours after venipuncture when using standard tubes for blood collection, leading to an increase in contaminating cellular DNA that may hinder analysis of circulating plasma DNA. Cell stabilization agents can prevent cellular lysis for several days, reducing the need for immediate plasma preparation after venipuncture, thereby facilitating the ease of blood collection and sample preparation for clinical research. However, the majority of cell stabilizing reagents have not been formally tested for their ability to preserve circulating plasma tumor DNA.

Design & Methods—In this study, we compared the properties of two cell stabilizing reagents, the cell-free DNA BCT tube and the PAXgene tube, by collecting blood samples from metastatic

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Disclosure of Potential Conflicts of Interest

B.H.P. is a paid member of the scientific advisory boards of Horizon Discovery, LTD and Loxo Oncology and has a research contract with Genomic Health, Inc. Under separate licensing agreements between Horizon Discovery, LTD and The Johns Hopkins University, B.H.P. is entitled to a share of royalties received by the University on sales of products. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies. All other authors declare no potential conflicts.

Author contributions

Conception and design: PVT, BE, JAB, BHP

Development of methodology: PVT, JAB, BE, RLC, DJZ, DC, BHP

Acquisition of data: PVT, JAB, BE, DAV, EY

Analysis and interpretation of data: All authors

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breast cancer patients and measuring genome equivalents of plasma DNA by droplet digital PCR. We compared wild type *PIK3CA* genome equivalents and also assayed for two *PIK3CA* hotspot mutations, E545K and H1047R.

Results—Our results demonstrate that blood stored for 7 days in BCT tubes did not show evidence of cell lysis, whereas PAXgene tubes showed an order of magnitude increase in genome equivalents, indicative of considerable cellular lysis.

Conclusions—We conclude that BCT tubes can prevent lysis and cellular release of genomic DNA of blood samples from cancer patients when stored at room temperature, and could therefore be of benefit for blood specimen collections in clinical trials.

Keywords

droplet digital PCR; circulating tumor DNA; plasma tumor DNA; cell stabilizing tube

Introduction

An emerging area of cancer research is the use of circulating cell-free DNA for the purpose of detecting mutations as a “liquid biopsy”, and as cancer specific biomarkers for early and late stage disease [1]. It has been known for several decades that cells, both normal and cancerous, shed DNA into the circulation, but only recently have technologies such as next generation sequencing (NGS) and digital PCR allowed for the sensitive and specific detection of individual molecules, which is needed to discriminate mutant cancer DNA from the large amount of wild type DNA present in plasma and other circulating fluids. Indeed, the use of plasma DNA for diagnostic purposes has already been extensively studied and implemented in maternal fetal medicine, where maternal blood harboring fetal DNA can now be used to diagnose genetic disorders in utero [2]. An inherent and often underappreciated problem with the use of plasma DNA for clinical medicine is the rapid lysis of white blood cells after venipuncture leading to cellular genomic contamination of plasma DNA. Prior studies have demonstrated that cell lysis after venipuncture can release genomic DNA from peripheral lymphocytes and other cells [3], leading to inaccuracies and potentially decreased sensitivity when assaying for rare mutant DNA molecules. Additionally, nucleases present in blood may also affect the integrity of plasma DNA. Thus, most studies have incorporated protocols that require preparation of plasma from whole blood within 1 to 2 hours after venipuncture [4], to ensure high quality plasma DNA and consistency in results. Unfortunately, such rigorous requirements greatly impede the ability to perform multi-institutional trials evaluating plasma DNA in various clinical settings due to pragmatic issues of sample handling and plasma extraction after blood draw in a timely manner.

Cell-free DNA BCT tubes (Streck, La Vista, NE) have been extensively tested and shown to stabilize cell membranes in whole blood and minimize cell lysis and subsequent release of cellular DNA into plasma. Although tested mostly for circulating fetal DNA in maternal blood [3, 5] a recent report demonstrates the ability of BCT tubes to prevent lysis of spiked breast cancer cells in blood samples, providing potential additional utility for this reagent in collecting circulating tumor cells (CTCs) [6]. However, to our knowledge, the use of BCT

tubes have not been formally tested for circulating plasma tumor DNA (ptDNA). In addition, other specialty tubes as well as standard reagents such as formaldehyde [7] are currently being used as cell stabilizing agents, but without analytic validation for plasma tumor DNA. Indeed, one report suggests that formaldehyde can hinder molecular analysis of plasma DNA [8]. Nevertheless, the validation and use of cell stabilizing reagents would greatly enhance current and future trials evaluating ptDNA as biomarkers of cancer burden, response to therapy and mutational status of tumors. Our laboratory has been evaluating the use of digital PCR for mutation detection in breast cancer patients with metastatic disease [9] and more recently in early stage breast cancers for use as biomarkers of minimal residual disease [10]. In order to expand our studies to multiple sites for future trials, we have evaluated BCT tubes in patient samples collected prospectively from metastatic breast cancer patients. We also tested another collection tube with a proprietary cell stabilizing reagent, the PAXgene Blood DNA tubes (Qiagen), used for preserving genomic DNA in lymphocytes by stabilizing cell membranes, though these tubes are not indicated for plasma DNA isolation. Our results suggest that BCT tubes can be used to preserve plasma DNA integrity for several days at room temperature, but that PAXgene tubes had inconsistent results with sample lysis as measured by total genome equivalents for a given locus using droplet digital PCR (ddPCR). The studies presented here suggest that BCT tubes can be used for transport of blood specimens to a central site for processing of plasma DNA, greatly facilitating analysis of ptDNA for clinical trials in oncology.

Materials and Methods

Ethics Statement

All human subjects research was performed under an IRB approved study, “Johns Hopkins Breast Cancer Program Longitudinal Repository”, by the Johns Hopkins Medicine Institutional Review Board #6 committee. Patients provided written informed consent to participate in the study, and the Johns Hopkins Medicine Institutional Review Board #6 approved the consent procedure.

Patients and Sample Collection

Patients diagnosed with metastatic breast cancer (n=10) were enrolled in an IRB approved repository study at The Johns Hopkins Sidney Kimmel Comprehensive Cancer Center. The protocol is approved to allow all patients with any breast anomaly to participate including those without a diagnosis of breast malignancy. To enrich for *PIK3CA* mutations, all breast cancer patients were diagnosed with estrogen receptor (ER)-positive breast cancer but had unknown *PIK3CA* mutational status of their tumor at the time of consent. Five 10 ml plasma samples were obtained per patient. Three blood samples were processed within 2 hours of phlebotomy: one collected in EDTA tubes (as a baseline control), one in PAXgene tubes, and one in BCT tubes. The two additional blood samples, one collected in PAXgene tubes and one in BCT tubes, were then stored at room temperature and processed seven days after phlebotomy. Plasma was prepared from tubes using a double spin procedure as previously described [9]. Cell-free DNA was extracted and purified from patient samples using the QIAamp Circulating Nucleic Acid kit (Qiagen), per the manufacturer’s protocol.

Droplet Digital PCR

Dual labeled (FAM or HEX) fluorescent-quencher hydrolysis probes were designed for *PIK3CA* hotspot mutations (E545K, H1047R) and their respective wild type loci as previously described [10]. The *EIF1AY* primer/probe set was purchased from Life Technologies (Cat. # 4400291) as a FAM labeled enumeration probe for the Y chromosome. Droplet digital PCR (Bio-Rad) was then performed as per the manufacturer's recommendation. Total DNA molecules, or genome equivalents, for each amplicon was quantified by the QX200™ Droplet Reader software according to the sum of FAM and HEX positive droplets. Results for each mutation analysis were recorded as the summation of eight replicates, creating a single meta-well for each sample. Although the QuantaSoft software can perform statistical analysis including fractional abundance and confidence intervals based upon Poisson statistics, for this study we did not utilize these parameters as we wished to show all raw data for comparison between samples (Table S1). Additionally, no pre-amplification was performed and only a limited number of genome equivalents were assayed per sample since we did not assess limits of sensitivity for this study, but solely differences between collecting tubes at varying time points.

Statistical analysis

All statistical analyses were performed using GraphPad InStat software (La Jolla, CA). A two-tailed paired *t* test was performed for each sample set. A *p*-value of less than 0.05 was considered significant.

Results

We initially sought to confirm prior studies demonstrating the ability of BCT tubes to preserve the integrity of circulating fetal DNA in maternal blood when kept at room temperature several days after venipuncture. As seen in Fig. 1A, blood obtained from a 7 month pregnant female with a male fetus was used to evaluate the ability of BCT tubes to preserve plasma DNA integrity after 7 days storage at room temperature. Using ddPCR with probes specific for the *EIF1AY* gene (on the Y chromosome) and a reference gene (wild type *PIK3CA*, exon 9), the number of genome equivalents as measured by wild type *PIK3CA* were roughly equivalent for plasma DNA extracted immediately after blood draw in EDTA tubes and in BCT tubes when blood was taken concurrently, but stored at room temperature for 2 and 7 days, being 59, 57, and 87 genome equivalents, respectively. In contrast, genome equivalents were an order of magnitude increased (669 genome equivalents) in plasma DNA samples from blood drawn in EDTA tubes that were left at room temperature for 7 days, consistent with lysis of lymphocytes and release of genomic DNA. However, the number of genome equivalents for *EIF1AY* were approximately equal between all 4 samples, indicating that for circulating fetal DNA, DNA stability was not adversely affected, though this could also represent compensation by release of genomic DNA from circulating fetal cells. These values demonstrate that in the absence of lysis, fetal DNA comprises approximately 6% to 10% of total maternal plasma DNA, which is consistent with the percentage of fetal DNA previously reported in maternal blood [11]. In addition, the ratio of *EIF1AY* to *PIK3CA* DNA was greatly altered due to the increase in

overall genome equivalents from cell lysis, skewing the fractional abundance of *EIF1AY* present in the EDTA sample that was stored at room temperature for 7 days.

The PAXgene tube is used as a cell stabilizing agent, but specifically to extract gDNA from lymphocytes and not for circulating plasma DNA. In principle, this tube could also be utilized for plasma DNA analysis, but to our knowledge they have never been tested for this application. We therefore tested whether these tubes could also be used for circulating fetal DNA analysis. We compared PAXgene tubes and BCT tubes from a separate volunteer, to obtain maternally derived circulating fetal DNA that was isolated from plasma prepared 7 days after blood collection, as well as EDTA tubes prepared within 1 hour of venipuncture. As seen in Fig.1B, plasma DNA from BCT tubes processed at day 7 had comparable genome equivalents to plasma from EDTA tubes harvested within 1 hour of venipuncture. In contrast, plasma DNA from PAXgene tubes processed at day 7 had an order of magnitude increase in reference genome equivalents (wild type *PIK3CA*, exon 9), though similar to EDTA tubes at day 7, genome equivalents of *EIF1AY* remained intact.

To determine if these results were also reproducible in cancer patients, we collected blood and completed analysis on 9 patients with metastatic breast cancer using ddPCR to assess for *PIK3CA* mutations and wild type genome equivalents. A tenth patient was enrolled, but the blood samples were unusable due to hemolysis, likely from needle shearing at the time of venipuncture. We analyzed plasma DNA derived from standard EDTA, BCT and PAXgene tubes prepared immediately post blood draw, and after one week's storage at room temperature for BCT and PAXgene tubes. Because this collection required a large ($5 \times 10 \text{ ml} = 50 \text{ ml}$) volume of blood at a single time point, we did not obtain additional blood samples per patient. We then assessed genome equivalents, including *PIK3CA* mutations, using ddPCR as previously described [10]. Similar to the results with circulating fetal DNA, we found that the total amount of DNA in plasma stored one week at room temperature in PAXgene tubes was greatly increased, showing on average a 37.14 ± 11.44 (mean and standard error) -fold increase in genome equivalents (Table 1). In contrast, the genome equivalents in plasma stored for one week in BCT tubes increased by 1.17 ± 0.14 (mean and standard error) -fold. Comparison of these means was statistically significant with $p < 0.0059$. At the extreme end, two patient samples demonstrated genome equivalents in day 7 plasma DNA collected in PAXgene tubes that were increased ~80 to 100-fold compared to the corresponding day 1 plasma DNA collected in the same reference tube (Fig.2). Comparison of average genome equivalents for each tube and time point to the corresponding day 1 timepoint revealed that only PAXgene tubes processed on day 7 had statistically significant increases in genome equivalents (Table 2).

Similar to NGS, the sensitivity of ddPCR for mutation detection is dictated by the “depth” of coverage or number of genome equivalents that is assayed for a given mutation, as well as the fidelity of thermostable polymerases. Therefore, lysis of white blood cells with subsequent release of genomic DNA could hinder the sensitivity of ddPCR since more genome equivalents would need to be analyzed. As with circulating fetal cells in maternal blood, lysis of CTCs may mitigate some of these concerns, though this adds even greater uncertainty and complexity in detection and quantification of ptDNA. Because the goal of this study was to compare cell membrane stabilization and not limits of sensitivity, we

analyzed only a finite number of genome equivalents per patient sample, and did not employ any pre-amplification steps. As shown in Table 1, four of nine patients in our study were found to have *PIK3CA* mutations in their ptDNA, with two E545K and two H1047R mutations identified. Because we did not perform “deep” ddPCR on patient samples, we only scored samples as positive for a given mutation if mutation positive droplets were found in all five collection tubes.

In patients with definitive *PIK3CA* mutations, day 7 BCT tubes consistently showed approximately equivalent genome equivalents to their baseline day 1 controls. Although PAXgene tubes did show preservation of genome equivalents in some patient samples (patients 2 and 4 for *PIK3CA* exon 9 genome equivalents, Fig.3), the two other patients with *PIK3CA* exon 20 mutation positive samples demonstrated significantly increased genome equivalents in their plasma DNA relative to baseline (patients 7 and 8, Fig.3), with most samples showing an order of magnitude increase. This was not due to technical issues with incubating DNA for several days in PAXgene tubes, as incubation of pure cell line genomic DNA inoculated into PAXgene tubes and processed at days 1 and 7 showed no difference in genome equivalents for exon 9 and exon 20 probes used for ddPCR (Table S2). Cell line genomic DNA was sheared per manufacturer’s instructions since small fragments are required for optimal incorporation into droplets, and more accurately reflects plasma DNA. Interestingly, and similar to our results with circulating fetal DNA, samples from PAXgene tubes that were lysed still had detectable levels of ptDNA similar to corresponding samples collected in EDTA or BCT tubes as determined by the presence of tumor-specific *PIK3CA* mutant DNA (Table S1). As mentioned above, this potentially represents DNA from lysed CTCs, though this cannot be definitively proven or disproven with the current analysis.

It should also be noted that there were instances of one or two mutant genome equivalents present in isolated tube specimens from either EDTA, BCT or PAXgene tubes (Table S1, patients 3, 5, 6, 7 and 8). These isolated events generally represent either extremely low percentages of mutant alleles or cross contamination/PCR artifacts, which can be distinguished by repeated sampling, and increasing the number of genome equivalents analyzed for each queried mutation. As stated previously, the purpose of this study was simply to compare plasma DNA integrity of cell-stabilizing reagents, and thus further analysis of these samples was not performed and was limited in some patients by the relatively small amount of plasma (per each sample tube) collected.

Discussion

The results presented in this study validate the use of BCT tubes for analyzing plasma DNA obtained from cancer patients’ blood samples stored for 1 week at room temperature. Although there was some variability with the use of BCT tubes, the vast majority of our assays still retained genome equivalents for a given amplicon that was similar to baseline controls. In contrast, our initial results with both EDTA and PAXgene tubes generally led to an order of magnitude or higher increase in genome equivalents after incubation of blood at room temperature for 7 days. These results strongly suggest that BCT tubes can be used for preserving plasma DNA integrity when blood is kept at room temperature. Although we validated the use of BCT tubes for only 7 days, the manufacturer’s specifications indicate

that blood can reliably be kept at room temperature for up to 14 days without compromise of plasma DNA integrity. In addition, other studies with BCT tubes have demonstrated that transport conditions using a range of ambient temperatures and simulated shipping conditions does not adversely affect plasma DNA [5, 12]. However, it should be noted that BCT tubes are validated only at temperatures between 6°C -37°C, and thus may require insulation for shipping under extreme weather conditions. Similarly, blood samples collected in BCT tubes should not be stored in a clinical specimen refrigerator and/or freezer since this induces lysis of cells in our hands (unpublished observations).

Although BCT tubes can clearly prevent lysis and release of cellular DNA, lysis of samples from either EDTA or PAXgene tubes did not appear to hinder the ability to detect circulating fetal DNA or ptDNA in our study. Whether this is broadly applicable is currently unknown due to the small sample size of our analysis, but it suggests that degradation of plasma DNA is not appreciable, for at least one week in room temperature when stored as whole blood. Alternatively, as mentioned above, the detection of circulating fetal DNA and ptDNA in lysed tubes may result in part from lysed circulating fetal and tumor cells, respectively, with subsequent release of genomic DNA into the plasma, which theoretically could compensate for any degraded DNA. This explanation seems less plausible, though we cannot prove or disprove this in the current study. Since the presence of CTCs is quite variable in patients with cancer [13], positive results from plasma samples obtained under conditions of possible cell lysis need to be interpreted with caution. Importantly, the fractional abundance of ptDNA (i.e. percentage of mutant DNA to total DNA for a given amplicon) becomes greatly altered when lysis occurs given the order of magnitude increase in wildtype genome equivalents as seen in our study. Given the uncertainty of CTC lysis and the potential for skewing of genome equivalents possibly affecting quantification and sensitivity of detection, we believe that EDTA tubes should only be used for plasma DNA analysis when samples can be prepared within 1 to 2 hours after venipuncture.

Our study also cautions against the use of general cell stabilizing agents for use in plasma DNA preservation. The PAXgene tube is specifically formulated for harvesting nucleic acids within cells kept at room temperature for up to 14 days after blood collection. The manufacturer's specifications also state that tubes can be kept refrigerated or frozen after venipuncture, followed by extraction of genomic DNA or RNA. Thus, lysis from cells and release of DNA into plasma may not be problematic for these applications, as the preservative used does not seem to adversely affect the quality of nucleic acids obtained and its use in downstream applications. On the other hand, the release of genomic DNA from cells can artifactually increase the number of cell free plasma genome equivalents, which may adversely affect absolute quantification of plasma DNA molecules. In theory, this could also affect sensitivity of a given assay, since more genome equivalents may have to be assayed to detect rare molecules found in plasma such as ptDNA. It is interesting to note however, that in our two patients with *PIK3CA* exon 9 mutations, genome equivalents at this locus were not increased in PAXgene tubes prepared at day 7, yet the same day 7 samples had increases in genome equivalents for the *PIK3CA* exon 20 locus. In fact, there was a general trend with PAXgene tubes of increased genome equivalents for the *PIK3CA* exon 20 locus compared to the *PIK3CA* exon 9 locus (Table 1). This was not a technical artifact as

ddPCR is a highly reproducible platform [14], and given our past expertise with the *PIK3CA* assay [10]. In addition, our results with cell line genomic DNA (Table S2), argue against a technical artifact. Thus, the reasons for this are unclear, but suggest that the amount of genome equivalents released from cells can vary in a locus specific manner, which could in turn lead to inconsistent results when quantifying DNA. Although this is not likely to be as problematic for ddPCR, allelic imbalances due to cell lysis could hinder analysis of DNA copy numbers for detecting cancer and fetal genetic anomalies.

In sum, our results confirm the analytic validation of Streck cell-free DNA BCT tubes for plasma DNA analysis of circulating fetal DNA and ptDNA. We also demonstrated that depending on the application, cell stabilizing products may not be suited for preserving the integrity of plasma DNA, noting the significant cell lysis that was observed in blood samples collected and analyzed in parallel with BCT tubes. These results provide the foundation for using BCT tubes to obtain blood samples for clinical trials in oncology that will facilitate the central collection and processing of specimens for plasma DNA analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

BCT tubes preserve plasma tumor DNA integrity at room temperature after venipuncture

PAXgene tubes do not prevent cell lysis at room temperature after venipuncture

BCT tubes can be used for plasma tumor DNA collection in cooperative trials

Figure 1. BCT tubes preserve circulating fetal DNA after 7 days storage at room temperature Droplet digital PCR was performed as described in the text using copy number probes for circulating fetal DNA with a probe for the Y chromosome (*E1F1AY* gene) and a reference locus (*PIK3CA* exon 9 wild type probe) using **A**) plasma DNA collected from a patient under standard conditions (EDTA), and after incubation of blood at room temperature in EDTA after 7 days and BCT tubes after 2 and 7 days as indicated, and **B**) from a separate patient under standard conditions (EDTA), and after incubation of blood at room temperature in BCT and PAXgene tubes after 7 days as indicated. Events refer to positive droplets for either *E1F1AY* or *PIK3CA* as indicated.

Figure 2. Genome equivalents in plasma DNA from BCT and PAXgene tubes processed after 7 days at room temperature

Genome equivalents were measured by ddPCR as per the text using probes for mutant and wild type *PIK3CA* for either exon 9 E545K or exon 20 H1047R mutations. Genome equivalents are shown for **A**) patient 6, assayed for the *PIK3CA* exon 9 locus, and **B**) for patient 9, assayed for the *PIK3CA* exon 20 locus. Events refer to positive droplets for either mutant or wild type *PIK3CA* exon 9 (E545 vs E545K) or exon 20 (H1047 vs H1047R) as indicated. DNA was extracted from 2 ml of plasma for each condition. Sheared cell line genomic DNA diluted at varying mutant to wild type ratios (1:1 shown) were used as positive controls.

Figure 3. Genome equivalents in plasma DNA from BCT and PAXgene tubes processed after 7 days at room temperature from patients with *PIK3CA* mutations

Genome equivalents were measured by ddPCR as per the text using probes for mutant and wild type *PIK3CA* for exon 9 E545K and exon 20 H1047R mutations. Genome equivalents are shown for **A)** patient 2, assayed for the *PIK3CA* exon 9 locus, **B)** patient 4, assayed for the *PIK3CA* exon 9 locus, **C)** patient 7, assayed for the *PIK3CA* exon 20 locus, and **D)** patient 8, assayed for the *PIK3CA* exon 20 locus. Events refer to positive droplets for either mutant or wild type *PIK3CA* exon 9 (E545 vs E545K) or exon 20 (H1047 vs H1047R) as indicated. DNA was extracted from 2 ml of plasma for each condition. Sheared cell line genomic DNA diluted at varying mutant to wild type ratios (2:1 and 10:1 shown) were used as positive controls.

Table 1

Mutational results and genome equivalent ratios for patients' plasma DNA.

Patient	Exon	Status	PAXgene tube Day 7 to Day 1 ratio	BCT tube Day 7 to Day 1 ratio
1	9	Wild type	11.82	2.07
	20	Wild type	26.96	2.59
2	9	E545K	0.87	1.45
	20	Wild type	25.94	0.81
3	9	Wild type	6.07	1.40
	20	Wild type	13.29	0.97
4	9	E545K	1.63	0.86
	20	Wild type	1.91	1.07
5	9	Wild type	16.53	0.74
	20	Wild type	22.60	0.86
6	9	Wild type	80.19	0.38
	20	Wild type	191.60	1.05
7	9	Wild type	23.93	0.46
	20	H1047R	33.22	0.48
8	9	Wild type	9.00	1.21
	20	H1047R	22.79	0.95
9	9	Wild type	70.81	1.80
	20	Wild type	109.33	1.86
Average $\pm$ SE M			37.14 $\pm$ 11.44	1.17 $\pm$ 0.14
Two tailed p-value			0.0059	

Table 2

Statistical comparison of average genome equivalents for patients plasma DNA in various collection tubes and time points.

Exon 9					
Tube	EDTA Day 1	BCT Day 1	PAXgene Day 1	BCT Day 7	PAXgene Day 7
Mean	294.89	305.00	244.78	297.33	4442.89
SD	305.74	355.01	270.85	303.81	5243.78
SEM	101.91	118.34	90.28	101.27	1747.93
n	9	9	9	9	9

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Exon 9				
	EDTA vs BCT Day 1	EDTA vs PAXgene Day 1	BCT Day 1 vs BCT Day 7	PAXgene Day 1 vs PAXgene Day 7
p-value	0.78	0.09	0.87	0.04

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Exon 20					
Tube	EDTA Day 1	BCT Day 1	PAXgene Day 1	BCT Day 7	PAXgene Day 7
Mean	127.78	136.00	134.67	151.11	3248.44
SD	114.55	175.55	165.44	188.40	2857.19
SEM	38.18	58.52	55.15	62.80	952.40
n	9	9	9	9	9

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Exon 20				
	EDTA vs BCT Day 1	EDTA vs PAXgene Day 1	BCT Day 1 vs BCT Day 7	PAXgene Day 1 vs PAXgene Day 7
p-value	0.77	0.76	0.32	0.01