**S2 Table. Comparison of results obtained in this study with previously published findings.**

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| **Topic** | **Observations Medina Diaz *et al.*** | **Observations in the literature** | **Ref.** |
| **Choice of blood collection tube** | * Performance of Streck cfDNA BCTs is comparable to K2EDTA and suitable for downstream liquid biopsy testing by BEAMing and Safe-SeqS
 | * Streck cfDNA BCT frequently applied for prenatal testing (K3EDTA collection tubes or cfDNA BCT recommended)
* Use of cfDNA BCTs advantageous if rapid sample processing not possible (NSCLC; Therascreen)
* Use of cfDNA BCTs could be of benefit for blood specimen collections in clinical trials (metastatic breast cancer: Droplet digital PCR)
* cfDNA BCT, CellSave, and K3EDTA tube effectively stabilize ctDNA and gDNA. cfDNA BCTs provides extended stability if kept at RT (metastatic breast cancer; Droplet digital PCR)

  | [1,2][3][4][5] |
| **Storage time influence (cfDNA and WBC stabilization)** | * Stable cfDNA yields and no detectable genomic DNA release from WBCs for up to 5 d at RT in cfDNA BCTs compared to 2 h RT storage in K2ETDA or cfDNA BCTs.
 | * Stable cfDNA and gDNA yields in cfDNA BCTs up to 7 d at RT, whereas gDNA increased 8-fold at day 14 compared to day one
* Stable cfDNA and gDNA yields in cfDNA BCTs up to 7 d at RT
* Stable cfDNA and gDNA yields in cfDNA BCTs up to 48 h at RT
 | [6][4][5] |
| **Storage temperature influence (cfDNA and WBC stabilization)** | * RT storage in cfDNA BCTs results in stable cfDNA yields and no detectable genomic DNA release for up to 5 d (compared to 2 h RT storage in K2ETDA or cfDNA BCTs)
* 3 - 5 d storage in cfDNA BCTs at 4 °C or 40 °C shows up to 10-fold increase of genomic DNA fragments compared to a 2 h storage in standard K2EDTA tubes
* 6 °C storage in cfDNA BCTs for 3 d shows a slight but significant increase in gDNA/cfDNA ratio compared to 3 d RT storage
 | * No difference in gDNA/cfDNA ratio for 4 °C storage compared to RT storage (6 h, K2EDTA tubes)
* No difference in cfDNA concentration for 24 h storage at 4 °C compared to 8 h storage at RT storage (K3EDTA tubes)
* No difference in gDNA/cfDNA ratio for 8 h storage at 4 °C compared to RT storage in K3EDTA tubes. cfDNA BCTs beneficial if storage at RT > 8 h (prenatal testing)
* cfDNA concentration does not vary significantly within 4-6 h following venipuncture at RT or at 4 °C (EDTA tube)
* Blood samples collected in cfDNA BCTs should not be stored in a refrigerator and/or freezer since this induces cell lysis (unpublished observations)
* cfDNA BCTs effectively stabilize ctDNA and gDNA for 6 h at 4 °C and RT. 2 out of 6 patients showed an increase of wild-type DNA at 48 h in cfDNA BCTs stored on ice, but not at RT
 | [7][8][9][1][4][5] |
| **Agitation influence (cfDNA and WBC stabilization)** | * 3 d permanent agitation of cfDNA BCTs does not alter cfDNA yields or genomic DNA release
 | * Agitation of blood samples for 3 h at RT causes a slight increase of cfDNA in EDTA tubes
* No significant change in cfDNA was detected in plasma if cfDNA BCT samples were shipped overnight at RT; Significant decrease in fetal DNA fraction was found in EDTA samples and cfDNA BCTs shipped overnight at 4 °C (prenatal testing)
* Shaking and shipment of blood in K3EDTA tubes shows a significant increases in gDNA, whereas no change is seen in cfDNA BCTs (RT, up to 24 h or RT 4 d shipment; prenatal field)
 | [1][10][6] |
| **Optical appearance of plasma fraction** | * Abnormal visual appearance of the plasma fraction during preparation of plasma from cfDNA BCT tubes shows strong correlation to plasma quality (broad interface cell layers, hemolytic plasma; increasing gDNA levels); visual appearance of plasma during preparation should be considered as a necessary quality control step
 | * n/a
 | n/a |
| **cfDNA amplifiability** | * cfDNA BCTs do not impair PCR amplifiability of cfDNA
 | * Reagent used in cfDNA BCTs has no effect on DNA amplification for storage up to 14 d at RT, whereas formaldehyde and glutaraldehyde treated DNA shows a time dependent decrease in DNA amplification indicating DNA damage
* cfDNA BCTs containing reagent does not affect downstream molecular analysis by PCR (Therascreen & Digital droplet PCR)
* cfDNA BCTs are not suitable for subsequent analysis of mSEPT9 with the Epi proColon 2.0 CE Early Detection Assay (the assay is a duplex PCR determining methylation of SEPT9)
 | [11][3–5][12] |
| **Mutation background in wild-type samples** | * No detectable effect of the cfDNA BCT preservative on the mutation background level of wild-type donor samples (BEAMing: 5 KRAS point mutations; Safe-SeqS: 5 c-KIT amplicons)
 | * n/a
 | n/a |
| **Detectability of mutant allele frequencies** | * Mutational load in CRC cancer samples was highly comparable between K2EDTA tubes stored for 2 h and Streck cfDNA BCTs stored for up to 3 d at RT
* The detection of low frequency spiked mutations (0.1%, 0.5%, 1%) is not impaired in samples stored in cfDNA BCTs compared to K2EDTA tubes
 | * Successful ctDNA recovery for mutation detection in NSCLC with cfDNA BCTs vs EDTA (Therascreen)
* Successful ctDNA recovery for mutation detection in metastatic breast cancer patients with cfDNA BCTs after 48 h or 7 d RT storage (Droplet digital PCR)
 | [3][4,5] |
| **Plasma volumes** | * Dropped slightly over 5 d RT storage in cfDNA BCTs
* Dropped >1 ml and was highly variable at 4 °C and 40° C storage for 3 d & 5 d in cfDNA BCTs
 | * n/a
 | n/a |

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