LBgard™ Blood Tubes for Liquid Biopsy

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Introduction

Liquid biopsy is key to enabling precision medicine, especially in oncology[1]. Critical biomarkers with prognostic and predictive values such as circulating tumor cells (CTCs) and circulating cell-free DNA (cfDNA) derived from tumors can be detected and analyzed from a simple blood draw[2-4]. Because these analytes are fragile, prone to degradation and present in extremely low quantities, proper preservation is required to ensure the accuracy of test results.

To ensure these critical analytes are protected in liquid biopsy samples, Biomatrica has developed LBgard™ Blood Tubes, which contain a preservative that stabilizes (1) CTCs for up to 4 days and (2) cfDNA for 7-14 days at 25°C. In addition, this unique Biomatrica stabilizer product limits blood hemolysis, which may interfere with downstream analyses (Fig 1).

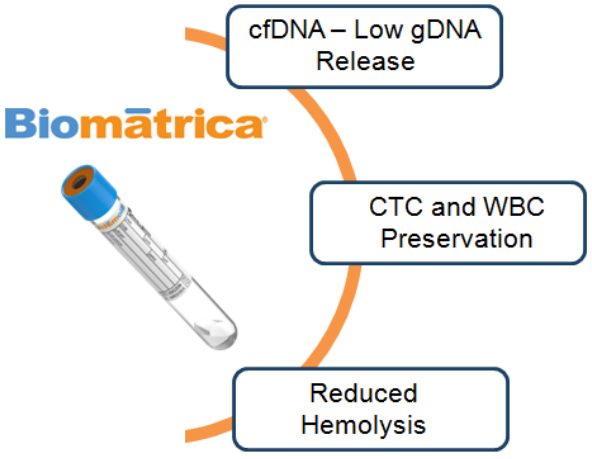


Figure 1. Preserving liquid biopsy in one blood tube.

Results

LBgard™ Blood Tubes stabilize cellular as well as plasma biomarkers in blood samples, thus enabling the characterization of CTCs and cfDNA in the same sample.

- Total plasma DNA is kept constant and genomic DNA release is inhibited for 7-14 days at 4°C, 25°C and 37°C (Table 1 and Fig 2). Fractional abundance of spiked-in mutant KRAS allele is maintained for 7 days as measured by droplet digital PCR (ddPCR) (Fig 3).
- Genomic DNA release in clinical samples from stage IV colorectal and breast cancer patients is inhibited for 7-14 days at 25°C (Fig 4).
- Hemolysis is inhibited for 7 days at 25°C (Fig 5).
- White blood cells (WBC) and spiked-in VCaP cells are stabilized for up to 4 days at 25°C (Fig 6-7).

Formulation	Day 0 Avg Yield (ng/ml plasma)	Day 0 Min – Max Yield (ng/ml plasma)	Day 7 Avg Yield (ng/ml plasma)	Day 7 Min – Max Yield (ng/ml plasma)	Fold Increase on Day 7 (Over Day 0)
EDTA	6.7 (n=5)	3.6 - 12.6	1227.9 (n=7)	950.7 - 1615.8	193.8 +/- 118.1
Streck	7.7 (n=3)	5.4 - 11.5	10.5 (n=6)	5.6 - 20.0	1.4 +/- 0.3
LBgard™	5.9 (n=6)	4.4 - 8.2	6.3 (n=6)	4.4 - 10.3	1.1 +/- 0.2

Table 1. The plasma DNA concentration is maintained by LBgard™ Blood Tubes over 7 days at 25°C.

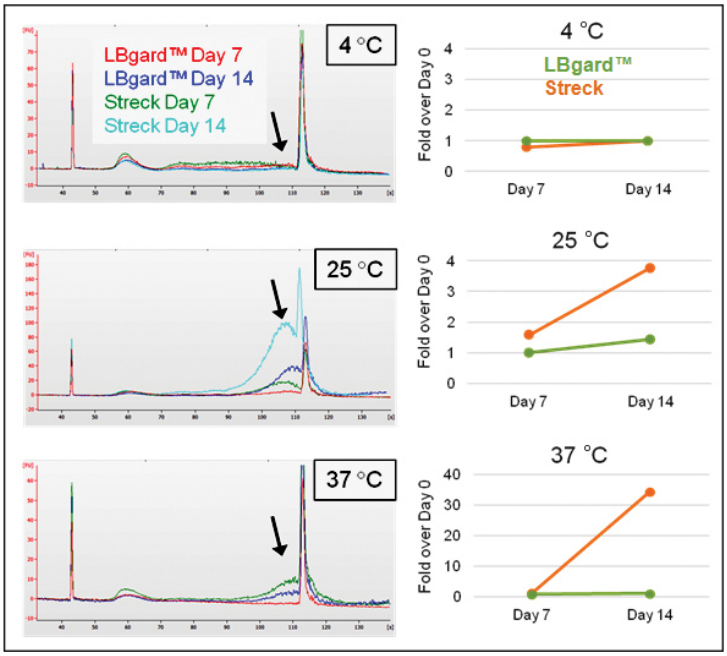


Figure 2. Genomic DNA release (black arrows) is inhibited (left panels) and DNA plasma concentration is maintained (right panels) by LBgard™ Blood Tubes over 14 days at 4°C, 25°C and 37°C.

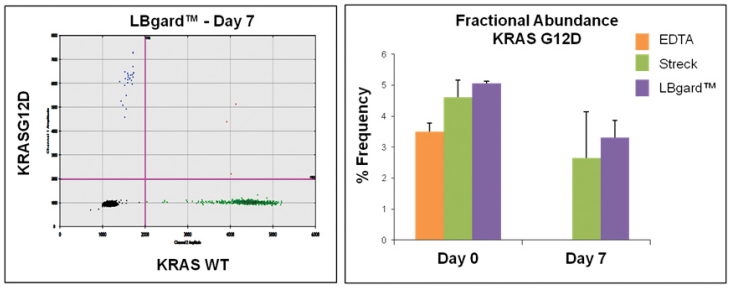
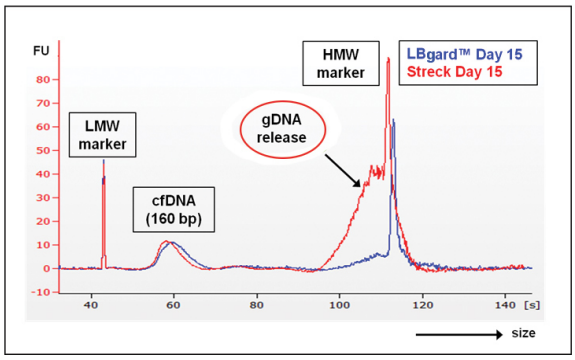


Figure 3. The fractional abundance of KRASG12D is maintained by LBgard™ Blood Tubes over 7 days at 25°C.



Individual Sample Yield (ng/ml plasma)			
Time-point	**Day 1/2**	**Day 7/8**	**Day 14/15**
Streck P1	11.5	10.5	ND
LBgard™ P1	8.1	8.1	ND
Streck P2	10.3	8.9	20.9
LBgard™ P2	7.6	8.3	10.1
Streck P3	59.7	73.6	60.3
LBgard™ P3	52.0	41.6	46.3
Streck P4	13.5	12.2	13.1
LBgard™ P4	13.0	10.8	11.0

Figure 4. Genomic DNA release is controlled by LBgard™ Blood Tubes in samples from stage IV breast and colorectal cancer (CRC) patients. A representative Bioanalyzer trace from one patient is shown (Top). cfDNA yield in ng/ml plasma for 4 clinical samples (P1: Breast and P2-P4: CRC). (Bottom) ND = Not Determined.

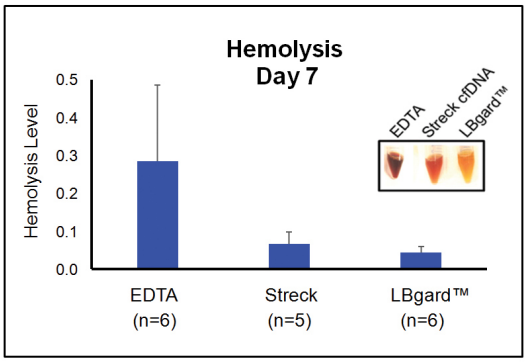


Figure 5. LBgard™ Blood Tubes control hemolysis in blood samples for 7 days at 25°C.

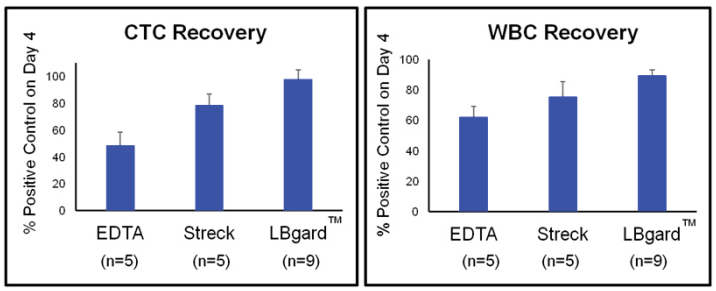


Figure 6. Spiked-in VCaP cells are preserved by LBgard™ Blood Tubes. VCaP cells (left) and WBCs (right) are recovered at 98% and 90% respectively, in LBgard™ Blood Tubes over 4 days at 25°C.

Conclusion

The LBgard™ Blood Tube, a new liquid biopsy preservation product, protects CTCs and cfDNA for downstream analyses from a single blood sample and enables liquid biopsy in routine cancer detection and management.

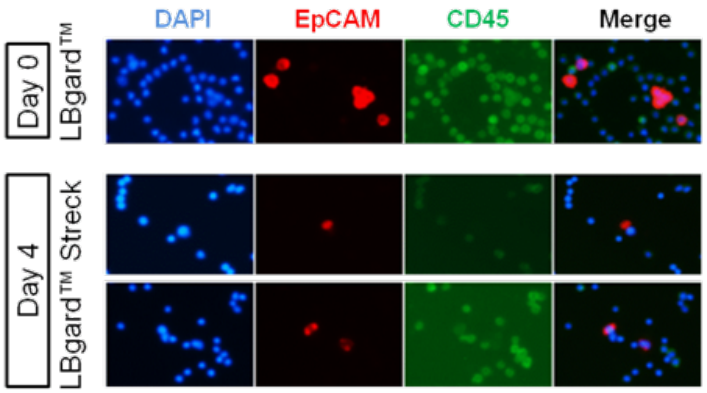


Figure 7. Cell morphology and protein biomarker expression are intact in both WBC (CD45) and spiked VCaP cells (EpCAM) for 4 days at 25°C.

Materials and Methods

cfDNA purification, quantitation and ddPCR analysis: Plasma was isolated from healthy donor blood collected in EDTA tubes (BD Bioscience), cfDNA BCT (Streck) or LBgard™ Blood Tubes and stored at indicated temperature and time-points. cfDNA was purified using QIAamp CNA Kit (QIAGEN) and quantified using Quant-iT dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific). cfDNA quality was assessed using High Sensitivity DNA Analysis Kit and 2100 Bioanalyzer (Agilent). Horizon cfDNA Reference (Horizon) was spiked into whole blood, and cfDNA was subsequently purified and analyzed by ddPCR using KRAS ddPCR™ Mutation Assay: KRAS p.G12Dc.35G>A, Human, and QX200 Droplet Digital PCR System (Bio-Rad).

CTC analysis by flow cytometry, immunofluorescent staining and hemolysis: VCaP cells were spiked into healthy donor blood collected in EDTA tubes (BD Bioscience), cfDNA BCT (Streck) or LBgard™ Blood Tubes and incubated at 25°C. On Days 0 and 4, mixtures were treated with RBC Lysis buffer (BD Bioscience). For flow cytometry, nucleated cells were stained with anti-CD45-FITC (Tonbo) and anti-EpCAM-PE (BioLegend) antibodies and quantified by absolute cell counts using the NovoCyte flow cytometer (ACEA). For immunofluorescent staining, nucleated cells were plated on glass slides, fixed and stained with DAPI (nuclear stain, Thermo Fisher Scientific), anti-CD45-FITC (Bio-Rad) and anti-EpCAM-PE (BioLegend) antibodies and imaged on the EVOS FL Cell Imaging System (Thermo Fisher Scientific). Hemolysis was measured by absorbance in a method described previously[5].

Clinical Samples: Stage IV colorectal cancer and breast cancer patient blood samples were collected in Streck cfDNA or LBgard™ Blood Tubes with IRB approval. Plasma cfDNA was isolated and analyzed as previously described.

References

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